# REPORT OF THE NORTHERN REGIONAL RESEARCH CENTER

May 1982



North Central Region
Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

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PROGRESS REPORT
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## INTRODUCTION

The Northern Regional Research Center, located at Peoria, Illinois, is one of the major research centers of the North Central Region, Agricultural Research Service (ARS), U.S. Department of Agriculture. Basic and applied research is conducted in the physical and biological sciences and in engineering. Northern Center scientists cooperate with representatives of colleges and universities, State experiment stations, research institutes and associations, industrial organizations, and with other Government agencies. Much of the cooperation is informal, but some work is conducted under cooperative agreements and memorandums of understanding. In addition, the Center's program is supplemented by research projects in foreign countries under Public Law 480 grants.

Providing scientific information for improvements in the American agricultural system is a major mission of NRRC research. These improvements inevitably benefit both consumers and farmers. For example, such research provides the basis for preserving and increasing food quantity, economy, quality, safety, and nutritive value. This research also offers opportunities to reduce the demand for foreign petroleum by generating technical knowledge for the production of alternative fuels, such as alcohol, and petrochemical-sparing chemicals from agricultural materials.

The subjects of research at this Center include corn, wheat, sorghum, oats, soybeans, and horticultural and special crops. Concentrating in the categories of plant, food, and biomaterials sciences, researchers create information leading to improvements in production, processing, and uses of these and other commodities. Among specific projects are ones designed to increase yields and decrease losses (notably by insect damage) both before and after harvest; to reduce processing costs and energy consumption and enhance food safety and quality; and to find uses for agricultural byproducts and residues.

A key research resource, the Agricultural Research Service Culture Collection (NRRL), is a world renowned repository of agriculturally and industrially important microorganisms. Reference cultures, cataloged taxonomic data, and professional expertise associated with this microbial germ-plasm bank have enabled NRRC to make vital contributions and to assume preeminent roles in mycotoxin research and in fermentation technology, including production of food ingredients and fermented foods such as tempeh. Because of the unique capabilities of a multi-disciplinary staff and the importance of the problem, research on mycotoxins has become one of the largest components of the Center's overall effort. This research plays a key role in protecting our food supply from these hazardous substances.

Center chemists, engineers, microbiologists, and physicists participate in joint projects with other ARS and SAES scientists conducting genetic and agronomic studies. Determination of processing and compositional characteristics of plant materials from botanical collections, breeding programs, and studies of soil and atmospheric variables is a major form of such participation. Another involves natural toxicants. Center scientists

provide analytical and biochemical information necessary to make sure levels of these minor constituents are not seriously increased in new varieties. This work, like the mycotoxin research, helps assure the safety and nutritional quality of our food supply.

Center scientists have added their weight to the growing emphasis on photosynthesis, nitrogen fixation, and plant tissue culture. Their novel biochemical, microbiological, and physical approaches complement longer standing studies by plant physiologists and thereby expand and diversify the total ARS attack in these high priority areas.

To take advantage of the resources and expertise available at NRRC, one of the two ARS energy centers, the Northern Agricultural Energy Center (NAEC), is headquartered here. The goal of NAEC is to discover, develop, and demonstrate technology which will permit agriculture to be energy self-sufficient on a net basis by 1990 under conditions that sustain productivity. The mission of the portion of the Energy Center program being carried out at NRRC is: (1) to develop innovative fermentative techniques emphasizing new microorganisms, new fermentation configurations, and use of substrates such as distressed grains; (2) to examine new approaches to substrate preparation and particularly investigate chemical, physical, and microbiological methods for conversion of cellulosic plant residues to sugars and thence to alcohol; (3) to evaluate the food and feed potential of nutrients isolated from fermentation residues and to develop methods to recover protein from these residues; (4) to expand the evaluation of plants as sources of hydrocarbons and to develop methods to separate and isolate hydrocarbon plant constituents; and (5) to evaluate vegetable oils as diesel fuels and develop liquid fuels derived from vegetable oil.

This report summarizes research of the Center during calendar year 1981 and lists publications and patents resulting from the research. The research summaries include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are available to those having a special interest in the development of public agricultural research programs.

This report was prepared at the Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604. Additional copies of the report and reprints of most publications can be obtained from the Northern Regional Research Center. A separate annual listing of publications and patents also is available.

## SELECTED ACCOMPLISHMENTS

Upgrading Corn Germ Flour as a Protein-Fiber Food Supplement. Extraction of corn oil from germ with super critical fluid $CO_2$ ($SC\text{-}CO_2$) gave excellent oil recoveries and has potential as an extraction process to replace conventional hexane extraction. The $SC\text{-}CO_2$ defatted flour rich in minerals, protein and fiber has an acceptable flavor score much improved over the commercial hexane defatted flour. Such $SC\text{-}CO_2$ extracted flour can be stored even under tropical conditions (100°F for 3 months) without deterioration of flavor. Oxidative rancidity is reduced significantly because $SC\text{-}CO_2$ flours contain very low levels of triglyceride oil and free fatty acids. Also, the peroxidase enzymes are denatured during extraction which reduces enzymic oxidation during storage. The flour contains low molecular weight proteins with balanced amino acid profile comparable to FAO standards. [See Biomaterials Conversion Laboratory (BC), B.2.]

A Starch-Borate Encapsulating Technique May Extend the Usefulness of Starch for Improving Efficacy of Pesticides. Addition of boric acid converts an alkali dispersion of starch and pesticide to a rubbery mass which is then broken into small particles by adding dry starch. Scanning electron micrographs reveal that the particles contain pesticide trapped in small cells. This processing technique is more efficient than the previous calcium or xanthate methods in that it allows much higher solids concentrations, improves pesticide recovery to nearly 100%, and retains water-soluble materials such as crop protectants. Dr. Marvin Schreiber (ARS, USDA), Purdue University, and other cooperating scientists continue to report new findings which support improved pesticide efficacy with starch encapsulated pesticides. When Dr. Schreiber surface applied starch-calcium encapsulated trifluralin in the fall, he found good weed control during the next growing season with little or no tillage. He also found better performance with shallow incorporation (1 in.) of starch-encapsulated pesticides than was found with the same pesticides as emulsifiable concentrates. These preliminary field studies suggest that starch-encapsulated pesticides can play an important role in programs designed to reduce soil erosion through reduced tillage. (See BC, C.3.)

Rapid Microwave Treatment for Salmonella-Free CSM. Recommendations have been made for a procedure utilizing microwave power to destroy salmonellae in contaminated corn-soy-milk (CSM). In a cooperative effort with a commercial supplier of CSM, it was determined that process temperatures necessary to destroy this organism in bagged CSM can be attained with a 60 Kw (2450 MHz) continuous microwave tunnel. Load levels of about 1 organism per gram could be reduced to nondetectable levels. It was also determined that heating and cooling must be carefully controlled in order to avoid serious nutritional damage. Operating parameters were established to carry out the treatment. This development provides a potential rapid means for guaranteeing absence of salmonellae in CSM. (See BC, D.1.)

High-Performance Gel Filtration Chromatography of Cereal Proteins. High-performance gel filtration (HPGF) has been successfully adapted for separating cereal proteins according to size and for analyzing cereal proteins to compare varieties. Though conceptually similar to earlier techniques, HPGF is fast (25 min per sample) and sensitive, has excellent reproducibility and resolution, and data are readily quantifiable. To date, HPGF has been used to determine protein molecular weights, to analyze heterogeneity of protein extracts, to compare protein classes, to detect interactions, to observe changes in molecular

folding and size due to chemical modification, and to analyze fragments produced by enzymic digestions of proteins. When used with new techniques for isolation of all proteins from less than single wheat kernel, HPGF can determine varietal purity and identity and can select unusual genotypes for breeding. [See Cereal Science and Foods Laboratory (CSF), A.5.]

A New Technique Has Been Developed for Determination of Phytate Using HPLC. This method allows determination of phytate as a single, well separated peak that is measured by a refractive index detector. This method has been proven for brans and now has been modified and extended for use on complex foods. High sensitivity, reproducibility, simplicity, and rapidity are important advantages of this new method. (See CSF, D.1.)

How Blue-Green Algae Might Contribute Fixed N to Wet-Land Agriculture. Research on the contribution of blue-green algae to nitrogen fertility of soils has led to isolation from a paddy soil of a blue-green alga capable of enhanced nitrogen fixation during formation of akinetes (sporelike forms). This finding has certain agronomic implications. Polypeptide granules in the akinetes provide a means for massively accumulating fixed nitrogen in paddys in a form that could be released subsequently for plant growth. In other words, the granules could constitute a substantial fraction of nitrogen in algal-rich paddy soils. Akinete formation in the algal strain is triggered by phosphate depletion. [See Fermentation Laboratory (F), A.2.]

Relationship of Yeast Genera Clarified. The major ascomycetous yeast genera Hansenula and Pichia are separated from each other only by their ability to assimilate nitrate, i.e., by the presence of a functional nitrate reductase. This criterion has been used for 50 years, but recent comparisons among species of the two genera showed high (68%) DNA relatedness between Hansenula mrakii and Pichia sargentensis. These data show the two taxa to actually belong to the same species and demonstrate nitrate assimilation to be of no value for separating species or genera. As a consequence, species in the two genera will be combined resulting in a more meaningful system of classification. (See F, C.1.)

Major Contribution to Reference Books of Importance to Agriculture. The worldwide reference book The Yeasts--A Taxonomic Study (3rd edition) compares all known yeasts and provides means for separating the more than 600 species which include many useful to food processing and industrial fermentations. Contributions from NRRC included chapters on the genera Citeromyces, Hansenula, Issatchenkia, Lipomyces, Pachysolen, Pichia, and Sterigmatomyces. Separation of species in this edition employs not only the standard morphological and physiological tests but also includes data from studies of genetics, ultrastructure, and DNA relatedness. The Fungal Community: Its Organization and Role in the Ecosystem, coedited at NRRC, is the first book to bring mycological ecology into the current framework of the ecological sciences. This 855-page volume outlines the research pathways being explored by mycologists and microbial ecologists and shows how this research relates to ecological theory. (See F, C.1.)

Relatedness of Lactic Acid Bacteria. The extent of genetic relationship of organisms can be determined by monitoring the intensity of interaction of their DNA. DNA interaction was determined between two newly described bacterial species, Lactobacillus amylophilus and L. amylovorus, and a broad spectrum of already established lactobacilli. Poor DNA interaction was observed between

the new species and the presently recognized species. Low interaction was also evident between the DNAs of L. amylophilus and L. amylovorus. The weak DNA interactions indicated that L. amylophilus and L. amylovorus were not highly related genetically to each other or to the established species, and hence, were two distinct and genuine species. (See F, C.2.)

Stimulating Mold Sporulation. Two fungi previously difficult to name to species because of their failure to sporulate when grown on usual laboratory media can now be more readily identified. Apophysomyces elegans and Saksenaea vasiformis grow luxuriantly on several media commonly used to identify fungi, but the isolates seldom or never form the reproductive structures used for identifying them. A technique was devised to enhance the production of such structures. Sporulation was stimulated by growing isolates on a rich medium followed by placing a small block of the medium permeated with hyphal growth onto water agar and incubating it at an elevated temperature. Rapid, accurate identification of these molds is necessary for safe handling when these potential pathogens are isolated from nature. (See F, C.4.)

Recombinant DNA. Partial cloning of the Aspergillus nidulans genome has been established in the lambda ($\lambda$) phage virus. Twelve kilobase fragments have been isolated by restriction of the isolated fungal DNA. The fragments were cloned into a $\lambda$ phage packaging system for establishment of a fungal genome bank. The viral clone bank can now be tested for the transformation of cellulase-degrading genes into a bacterial/yeast system for expression. (See F, C.5.)

Viruses Decreased by Fermentation. In the continuous fermentation of corn with feedlot waste liquid, five bacterial virus populations were substantially reduced in numbers. After 5 days' fermentation, the output contained less than 0.1% of the viruses added. With four of the viruses added initially at $10^8$-$10^{10}$ viruses/g. total material, the viral numbers decreased 90% in 11 to 18 hours; while Ø-6 virus was totally killed in 5 minutes. The data support strongly the use of a directed fermentation to decrease potential disease-producing viruses in animal wastes. (See F, D.1.)

Information on Tofu Properties May Help Small Business Operators. With the rapid development of tofu market in this country, the need for quantitative information on making tofu has become more apparent so that uniform good quality products can be produced. Our study shows that both the quality and the quantity of tofu are affected by coagulation conditions such as type and concentration of coagulants, temperature of the soybean milk, and the mode of mixing, as well as the pressure applied to remove the whey. Thus, one can manipulate the coagulation conditions to achieve the desirable products, but one must observe the selected set of conditions to obtain uniform products. For maximum nutritional value, soybean slurry should be boiled for 10-15 minutes. The most suitable concentrations of coagulants in the soybean milk are 0.02-0.04 M. Calcium sulfate ($CaSO_4 \cdot 2H_2O$) is preferred because it produces tofu of high bulk weight, high nitrogen recovery, smooth and fine texture. (See F, F.1.)

Transmission of Aflatoxins in Tissue of Cows Fed Aflatoxin $B_1$. Two Holstein cows (1200 pounds) were given 0.35 mg/kg $B_1$/kg cow per day for 3 days; cow one was sacrificed 24 hours after the last dose and cow two 6 days after the last dose. Aflatoxins $B_1$ and $M_1$ (which is produced by the cows from $B_1$) were detected in all 16 animal organs and other tissues of cow one except thymus.

Highest levels of $B_1$ and $M_1$ were found in the liver (7.1 ng $B_1$/g and 6.1 ng $M_1$/g) and kidney (1.3 ng $B_1$/g and 56.6 ng $M_1$/g) of cow one which is similar to results obtained on 300-pound steer. Aflatoxins $B_1$ and $M_1$ (0.02-0.5 ng/g) were found only in the liver, kidney, and urine of cow two, indicating that aflatoxins are eliminated from the tissues of cows when contaminated feed is withdrawn. Urine might be used to determine when tissues are aflatoxin-free to the point that the cattle are marketable. (See F, G.2.)

Five-Year Study of Wheat and Corn in Virginia for Aflatoxin, Zearalenone, and Ochratoxin Occurrence. In a 5-year (1976-1980) study on 100 samples each of wheat and corn collected by the Federal Grain Inspection Service, no aflatoxin, zearalenone, or ochratoxin A were found in any wheat, indicating the wheat was not subject to contamination by these mycotoxins. None of the corn had detectable zearalenone or ochratoxin A. However, aflatoxin was detected in corn harvested every year, but average levels (21-137 ng/g) and incidences (36-86%) varied greatly. Other samples of freshly harvested corn collected by the Statistical Reporting Service in 1978 and 1979 contained lower average levels (13, 36 ng/g) and incidences (12%, 21%). (See F, G.2.)

Successful Collaborative Study Results in Approval of Method for Determining Aflatoxin in Animal Tissues. The NRRC method for the determination of aflatoxin in edible tissues was tested in an international collaborative study by 14 scientists from England, France, Germany, Japan, Poland, South Africa, The Netherlands, and the United States. Based on the results of this study, the NRRC method was approved by the Association of Official Analytical Chemists and the International Union of Pure and Applied Chemistry for monitoring meats in the world market. (See F, G.2.)

Aflatoxins in Airborne Dusts Collected in Personal Samplers from Contaminated Corn at Harvest and at Elevators. In 1980, airborne dust samples were collected with personal and total samplers during harvest and at an elevator in Georgia. On the farm, the average aflatoxin levels in dusts from personal samplers was 18,500 ng/g and from total dust samples, 3000 ng/g. The aflatoxin level in bulk corn harvested during dust collections was 1600 ng/g. High toxin levels in airborne dusts can be accounted for by spores of the Aspergillus flavus group. In a study of aflatoxin in the spores of nine species of the Aspergillus flavus group, the average toxin level was 103,000 ng/g. At the elevator, the average aflatoxin level in bulk corn was 153 ng/g; in airborne dust samples collected by total samplers, 1200 ng/g; and in dust collected in personal samplers, 668 ng/g. (See F, G.2.)

Aflatoxin Ammoniation Pathway Proposed. Ammoniation of aflatoxin-contaminated corn at atmospheric pressure provides an effective means for destroying this mycotoxin, but the fate of the aflatoxin is not known. In an approach designed to elucidate the chemical events of this process, 5,7-dimethoxycyclopentenon [2,3-c]coumarin, which lacks only the bisdihydrofuran rings of aflatoxin $B_1$, was synthesized and its ammoniation products identified. When this coumarin is adsorbed onto diatomaceous earth and ammoniated under conditions resembling the corn-decontamination process, the lactone ring opens and undergoes oxidative decarboxylation to produce a diketone. This intermediate then loses the cyclopentene ring to give 3,5-dimethoxyphenol. The phenol, although stable to ammonia in solution, is oxidatively ammoniated when distributed over a large surface area to produce a multitude of products. One of these has been identified as a phenoxazone. The reaction mixture is very complex, and the identifiable products are formed in less than 10% yield. Aflatoxin $B_1$ would

be expected to degrade in a manner paralleling the model coumarin. (See F, G.3.)

Vomitoxin Produced for Biological Evaluation. Ample quantities of vomitoxin (deoxynivalenol) (3.5 g; current price is approximately $6,500 per g) have been prepared by solid substrate fermentation on corn or rice with Fusarium graminearum NRRL 5883. Vomitoxin has been distributed to veterinarians and animal scientists in the U.S., Canada, and Austria for evaluation in small laboratory animals, chickens, swine, and turkey poults. Collaborators at the Department of Poultry Science, North Carolina State University, have found that vomitoxin administered to broiler chicks via crop intubation causes frank hemorrhaging throughout the carcass, widespread deposition of urates, disturbance of the nervous system, and irritation of the upper gastrointestinal tract. Vomitoxin appears to be the first mycotoxin reported to cause the hemorrhaging characteristic of the chicken hemorrhagic anemia syndrome. (See F, G.3.)

Longevity of Aspergillus flavus in Corn. Aspergillus flavus and A. parasiticus are the two fungi that produce aflatoxins in corn both in the field and in storage. Because corn sometimes is stored for several years before it is used, one can ask how long can these fungi survive in kernels of corn. Twenty-one samples of white corn, assayed for aflatoxin and A. flavus in 1972, were reexamined. In all samples containing A. flavus in 1972 the fungus was still present, but the counts were always less than in 1972. The number of A. flavus propagules was reduced from 73 to 7.8%. Reduction in the numbers of A. niger and species of Fusarium and Penicillium was also found. (See F, G.4.)

Cleistothecia of Eupenicillium ochrosalmoneum Form Naturally Within Corn Kernels. The toxigenic mold Eupenicillium ochrosalmoneum Scott & Stolk, isolated primarily from cereal products and foodstuffs, formed cleistothecia (the sexual stage) within insect-damaged and fungus-infested corn kernels from fields near Tifton, Georgia. The cleistothecia were exposed by removing sections of the kernel pericarp. Cleistothecia contain ascospores and represent the sexual stage of the fungus life cycle. This is the first time that any mold has been found to produce cleistothecia within the seed of a vascular plant. The implications for agriculture are significant because cleistothecia would thus represent a principal source of fungal inoculum where cereals are grown. (See F, G.4.)

Cuphea Development Underway. Although previous NRRC research had shown seed oils of various Cuphea species to be excellent sources of lauric acid and other medium chainlength fatty acids, interest in further development has only recently become eminent. During the past few years, supply and price problems developed in the traditional source of these fatty acids, namely coconut oil, and combined efforts of private enterprise, government, and universities have resulted in an international effort to bring Cuphea to crop status. Close cooperation between BARC, NRRC, University of California at Davis, University of Göttingen (West Germany), Kent State University, and several chemical companies, has already led to seed collections from Mexico and Brazil, chemical and agronomic evaluations, and germplasm evaluation and improvement. Field trials have already been started in Germany and Brazil, and will be attempted at Davis, CA, in 1982. [See Horticultural and Special Crops Laboratory (HSC), B.1.]

Development of Harringtonine/Homoharringtonine. The National Cancer Institute (NCI) has recently started clinical testing of homoharringtonine as the final step toward elevation to drug status for this anti-cancer compound. Harringtonine and homoharringtonine are alkaloids which were isolated and characterized in the HSC Laboratory several years ago, and showed activity in several experimental tumor systems. Enough product was extracted from plant material to complete preclinical testing. Although pathways were found at NRRC and other laboratories to synthesize these alkaloids, supplies remained short. In the meantime, these compounds underwent a considerable period of development in the People's Republic of China, where natural sources are relatively abundant. When additional supplies became available, NCI completed recently its toxicology studies, and started the clinical studies in mid-November. Preliminary results may be expected as soon as mid-1982. (See HSC, B.2.)

Bicarbonate Control of Light Reactions in Photosynthesis. Work during the past year in collaboration with A. Stemler (University of California, Davis, CA) demonstrated a very slow chlorophyll a fluorescence decay in single-flashed bicarbonate-deficient chloroplasts. This slow fluorescence decay component, which is enhanced two- or threefold by bicarbonate depletion, corresponds to a retarded $Q^-B \rightarrow QB^-$ reaction, one of the first steps in electron transfer from the photolysis of water. The discovery that low bicarbonate levels inhibit $Q^-$ reoxidation without affecting photosystem II reaction centers, suggests a control mechanism that can balance reducing power and ATP output of the light reactions. This site of action, at a primary quinone charge carrier, is where many herbicides are believed to act. (See HSC, C.2.)

Corn Yield Possibly Linked to Chloroplast Structure. Chlorophyll b/a and xanthophyll/carotene ratios in low-yielding corn varieties of the 1930's are lower than those in contemporary high-yielding varieties. High chlorophyll b/a and xanthophyll/carotene ratios are associated with increased light-harvesting pigment and grana formation. Such changes can be related through the work of others with spinach and algae to increased levels of photosystem II relative to photosystem I. (See HSC, C.2.)

Nitrogen Isotope Ratio Analysis Now To Be Automated. An automated system has been devised which generates nitrogen gas from ammonium chloride, after reacting with lithium hypobromide, and inlets the gas into a mass spectrometer. The mass spectrometer then analyzes the gas for $^{14}N:^{15}N$ ratios. These ratios are used by soil scientists in their fertilizer tests. The system runs under computer control which allows samples to be analyzed around the clock without human presence. Data are processed by computer and printed for distribution to cooperators. (See HSC, F.1.)

Improved Method for Analysis of Natural Toxicants in Carrots. Carrot roots (and parsnips) can now be accurately analyzed for three toxicants, the hallucinogenic myristicin and two acetylenic alcohols, falcarinol and falcarindiol. The high level (38-268 ppm) of falcarindiol in carrots produced in Wisconsin was unanticipated. The toxicology of this compound is largely unknown, but from preliminary data it appears to be more toxic (acute) than falcarinol. (See HSC, G.1.)

Crambe Meal Approved for Beef Cattle Feed. Food and Drug Administration published the final rule permitting the use of crambe meal as a protein supplement in the feed of beef cattle, not to exceed 4.2% of the total ration. This makes it more attractive economically to grow high-erucic-acid crambe

domestically because the byproduct meal can generate credit as livestock feed. (See HSC, G.2.)

Isolation and Purification of Soybean Peroxidases. Peroxidases can contribute to deteriorative changes in flavor, texture, color, and nutritional quality in both raw and processed food products. Peroxidases, in an oxidasic reaction, can utilize molecular oxygen as an acceptor with subsequent generation of free radicals. These radicals, in turn, can propagate the decomposition of many compounds including polyunsaturated fatty acids and endogenous phenolic constituents with possible generation of off-flavors. To test this hypothesis for the generation of off-flavors in soybeans, methods for isolating and purifying soy peroxidases were developed. In the first phase of this research, soy peroxidases were purified 628-fold with a yield of 4% and some of their basic properties were established. [See Oilseed Crops Laboratory (OC), A.3.]

Analysis of Soybeans and Soybean Protein Products for Cyanide. Since soybeans are a major item of the diet in certain countries such as Japan, which imports much of its supply from the United States, there is concern about levels of minor components such as cyanide compounds which might have antinutritional effects on a long-term basis. Procedures were therefore investigated for determining levels of cyanide in soybeans. Only 0.07 to 0.3 μg CN was found per gram of soy protein product, and no cyanogenic compounds were detected. These results indicate that cyanide and cyanogenic compounds are of little nutritional significance in soybean products. (See OC, A.3.)

Soybean Seed-Coat Structure. Examination of 33 soybean varieties with a scanning electron microscope revealed a wide diversity in seed-coat structure. This previously unsuspected diversity may assist soybean breeders in developing varieties with seed coats having a greater resistance to cracking and to invasion by microorganisms during adverse storage conditions. Such improvement would upgrade the quality of soybeans and would benefit farmers, soybean handlers, processors, and consumers of soybean foods. (See OC, A.4.)

Isoflavone Analysis of Soybeans and Soybean Protein Products. A procedure was developed for the separation and quantitation of soybean isoflavone glycosides and isoflavone aglycones using high-pressure liquid chromatography with mild solvents and reverse phase packing. Dehulled, defatted flours contain the following mean isoflavone contents (mg/100 g): daidzin 61.7, glycitein glucoside 12.9, genistin 119.8, daidzein 32.8, genistein 26.7. The same isoflavones were found in soybean protein concentrates and isolates, but in decreased amounts. The isoflavone content of soybeans is variable and depends on variety, location, and crop year. Extracting the oil from soybeans did not remove the isoflavones or the isoflavone glycosides. Most of the isoflavones are concentrated in the hypocotyl, and the isoflavone content of hulls is quite low. (See OC, A.4.)

Dietary Fat: Metabolic Fate of Fatty Acids Isomers. Tissue analysis and isotope tracer studies with human subjects were used to assess both the short and long-term nutritional impact of fatty acid isomers in dietary fat. Results show both preferential incorporation and selective discrimination of the isomers for various plasma and tissue lipid classes. Each isomer was metabolically unique depending on its double bond configuration and location. Preferential uptake of cis-12-octadecenoic acid by phospholipids was 5 fold higher than for any other isomer and resulted in a significant decrease in the polyunsaturate to saturate ratio. No significant accumulation in plasma,

heart and liver lipids occurred which suggest the various isomers do not disrupt the activity of the numerous enzymes involved in lipid metabolism. (See OC, D.1.)

Fermentation Ethanol from Wheat Straw Hemicellulose. An autohydrolysis technique was employed to produce crude xylose directly from wheat straw. The crude 5-carbon sugars solution was directly fermented to ethanol by the yeast, Pachysolen tannophilus. Hydrolysis converted 60% of wheat straw pentosans to fermentable xylose by treatment with 5% sulfuric acid at 100°C for one hour. Addition of Pachysolen yeast cells at $10^9$/ml converted all of the xylose to products in four days with ethanol concentration of 1-1.2%. [See Northern Agricultural Energy Center (EC), A.2.]

High Performance Liquid Chromatography Alcohol Fermentation Broth. High performance liquid chromatography (HPLC) is a powerful tool for studying the effect of variables on the course and products of yeast fermentation of grain and other agricultural commodities to produce fuel alcohol at lower cost. In the past, several time-consuming, different analytical methods requiring special equipment were needed to establish glucose, xylose, glycerol, acetic acid, acetaldehyde, lactic acid, and ethyl alcohol contents of fermentation broths at different stages. Using a short column containing an ion exchange and reverse phase absorbance resin, assay of these major fermentation products can be obtained from broth filtrates in 20 minutes and monitored by refractive index difference. Using this procedure has not only resulted in great economic savings but it has permitted the demonstration of fermentation parameters which can minimize formation of byproducts and maximize ethanol production. (See EC, A.4.)

Low-Energy Preservation of Distillers' Feed Grains. Distillers' feed grains are valuable fermentation byproducts that are recovered wet and are extremely perishable. Sorbic acid, potassium sorbate, propionic acid, ammonia, and carbon dioxide were compared as preservatives and as alternatives to energy-intensive, high temperature drying of wet grains. Sorbic acid was the most effective chemical agent tested. Storage time of treated grains was extended over one month. This discovery offers the livestock producer more flexibility in using this highly perishable material in his feeding program. (See EC, A.4.)

Recycling Distillers' Solubles Solution Cuts Costs and Energy Consumption in Alcohol Production from Grain. A major cost and source of energy consumption during alcohol production via fermentation of grain is the evaporative concentration and drying of distillers' solubles (stillage waters). After alcohol is distilled from the beer, the bulk of the insolubles are removed by screening and centrifugation leaving 2 to 4% of solids in the stillage waters. We have found that when centrifuged effectively, the clarified stillage waters can be fully recycled several times for use in mashing and fermenting ground corn in subsequent runs without imparing alcohol yields. The dissolved solids content rose to 9% following recycling 9 times but only one tenth the normal volume remained for concentration. By recycling the stillage waters 9 times, the cost of producing alcohol on a commercial scale can be reduced by approximately 10¢ per gallon due to energy savings on equipment and water. (See EC, A.4.)

Alternative Fuels Technology Transfer. A series of workshops, seminars, and lectures sponsored by the Northern Agricultural Energy Center (NAEC), the

Cooperative States Research Service, the Extension Service, and Purdue University has resulted in clarification of the state-of-the-art in alternative fuels and of the research directions that need to be undertaken to help meet the USDA goals of equalization of agricultural energy output and input by 1990 and of providing a surplus of farm-generated energy by 2000 and of providing sources of fuel for crop production under emergency conditions. Proceedings of the workshops and seminars have been widely distributed and are noteworthy for their comprehensiveness and timeliness of information. (See EC, A.5.)

## BIOMATERIALS CONVERSION LABORATORY

W. M. Doane, Chief

Research Leaders: R. A. Anderson, R. J. Bothast, G. E. Hamerstrand, and F. H. Otey

### A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

1. Ion Transfer in Photosynthesis (Cooperative Agreement - University of Notre Dame)

   a. Specific Objective: Develop sensitive methodologies for quantitating, in real time, the concentration of hydrogen ions within the entrapped aqueous compartment of reconstituted vesicles formed from the photosynthetic membranes of plants and bacteria.

   Progress: The water-soluble fluorescence probes 8-hydroxy-1,36-pyrenetrisulfonate (pyranine) and fluorescein isothiocyanate-dextran (FITC-dextran) were found to be satisfactory fluorescence probes of intravesicular aqueous pH in reconstituted systems. Studies on the kinetics of intravesicular pH changes occurring during photosynthetic reactions such as $O_2$ evolution and ATP formation were initiated in reconstituted systems and in subchloroplast particles.

   Publications:

BELL, D. H., J. M. GOULD, AND L. K. PATTERSON. The Real Time Kinetics of Luciferase Inactivation by Pulsed Ionizing Radiation. Radiation Res. In press (1981).

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### B. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Basic Studies of Physical Properties of Soy and Cereal Based Food Ingredients (E. B. Bagley)

   a. Specific Objective: Investigate isopropanol (IPA) as an alternate solvent to hexane for extraction of soybeans.

   Progress: The oilseed extraction plant was remodeled to accommodate the IPA process for extracting oil from soybeans and includes the installation of coalescers and a phase separator. A series of pilot-plant experiments in which the IPA concentration is varied from 85.0 to 90.5% w/w and solvent-to-meal ratios of 1.5 to 3.0 have been investigated. The IPA phase is separated from the miscella by cooling

and coalescing rather than by the conventional evaporation procedure required with the hexane process. IPA recovered this method was recycled to the extractor to account for more than 75% of the solvent requirement.

b. Specific Objective: Determine the viscoelastic properties of doughs by eccentric rotating disc (ERD) rheometer and relate to components and final food properties.

Progress: Measurements of the dynamic storage and loss moduli G' and G", of starch dispersions prepared at temperatures in the gelatinization range have been carried out using the eccentric rotating disc (ERD) rheometer. These dispersions were in the water limited concentration range (doughs) and combined with the viscosity studies under 20520-028, in which water was not limiting, provides information over a broad concentration range. The work has served to bring into focus some very basic problems in the characterization of doughs and the evaluation of component interactions. For example, the relationships between the complex viscosity (calculated from G', G") and the steady shear viscosity for these dispersions is not yet established for certain. Further, studies with gluten added to the starch dispersions at low test strains showed less dependence of properties on gluten than expected. In examining the material response at larger strains in the ERD mode, however, we uncovered some unexpected results including yield points and time effects. Cooperative work with University of Michigan has been undertaken to resolve some of these results.

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2. Fundamental Studies on Separation of Starch, Protein, and Lipid of Corn (R. A. Anderson)

a. Specific Objective: Evaluate supercritical $CO_2$ extraction process for preparing edible corn germ flour with improved flavor and shelf life.

Progress: An acceptable food protein supplement was obtained by supercritical $CO_2$ extraction of oil from dry milled corn germ. The defatted meal has good flavor and is stable during accelerated storage studies at 100°F for 6 months. It was found that the SC-$CO_2$ extraction process inactivates peroxidase enzyme which could account for the good storage stability. Off-flavor constituents, such as bitter phenolic acid derivatives, are also extracted from the meal by SC-$CO_2$. This helps to upgrade taste and flavor as compared to hexane extracted meal.

b. Specific Objective: Evaluate the SC-$CO_2$ extraction of cereal bran and determine the effect on taste.

Progress: No improvement was found in the initial taste of SC-$CO_2$ extracted wheat bran as compared to hexane extracted bran. However, $CO_2$ did extract more non-triglyceride lipid material than did hexane which might improve both flavor stability and properties of doughs into which the bran could be incorporated as a fiber source.

c. Specific Objective: Determine oil recoveries by supercritical $CO_2$ extraction of wet-milled germ obtained from high-oil corn mutants and evaluate residual flour as a food product.

Progress: Oil from wet milled corn germ was obtained by SC-$CO_2$ extraction of germ at three moisture levels, 3, 14, and 26%. Oil recovery was strongly dependent on initial moisture of the germ, being 45%, 41%, and 31% for the three moisture levels. This low recovery may be a purely physical effect. As $CO_2$ passes through the germ, it carries the moisture chromatographically from the top to the bottom of the germ column. As the moisture in the bottom third of the column rises, the germ becomes a glutinous, packed, impermeable mass and the oil extraction rate drops markedly. Removal of the germ from the extractor, remixing and then pin-milling, restores the germ to a physical state which permits continued extraction of oil by $CO_2$ at the initial rates observed. By this second extraction residual oil in the meal could be reduced to 0.8%. Taste tests showed that SC-$CO_2$ extraction also

removed some bitter and astringent flavor components which are not removed from a hexane defatted meal. Flavor stability of twice-extracted meal during storage at 100°F for 2 months was excellent.

d. Specific Objective: Reduce energy requirements in the corn milling industry.

Progress: Dry milling studies on the relation of kernel hardness to dry milled product characteristics were performed on 10 different corn hybrids, which represent about 80% of the yellow dent corn grown in Illinois. All of the hybrids showed extreme hardness, with less than 1% breakage (Stein breakage test), and good kernel soundness was exhibited with stress crack counts of 4% or lower. Dry milling of these corns gave degermer thruputs (measurement of power needed to grind the corn) ranging from 93 to 197 lb per hour while yields of prime products (grits, low fat meal, and flour) varied from 54 to 64%. There appears to be little or no correlation between hardness and milling yields in these samples.

The effects of varying temper moisture additions and temper times were investigated in a short corn dry milling flow. Over a range of temper moistures of 13 to 24% and temper times of 15 min to 18 hours, only minimal milling effects were noted. Yields of -3½ + 7 grits ranged from 67 to 70%, and fat contents of the grits varied from 1.0 to 1.3%. The attached hull counts were lowest at 22% using the conventional 3-step temper system. Hull counts varied up to 88% when lower moisture and shortened temper times were used.

Pilot plant quantities of intrinsically labeled $^{65}Zn$ corn products, i.e., corn flakes, grits and flour, were prepared for human feeding tests in cooperation with the Cereal Science and Foods Laboratory, NRRC, and the Human Nutrition Laboratory, Grand Forks, ND. Cooperative work with USDA, OICD scientists on prototype products from millet continued.

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3. Development of Mycostatic Systems to Permit Safe, Low-Energy Grain Drying (R. A. Anderson)

a. Specific Objective: Maintain communication link with farmers and others concerning usage of the Trickle Process for preserving and ambient air drying of high-moisture corn.

Progress: Although the project was terminated on September 30, 1981, and a final report submitted, numerous mail and telephone inquiries were handled during the period. It appears that many farmers will be using the process during the 1981 harvest because of the increased amount of high-moisture corn resulting from late planting and adverse climatic conditions in certain areas of the corn-growing regions of the U.S.

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## C. BIOMATERIALS SCIENCE

1. Plant Component Separation and Physical Characterization (T. P. Abbott)

   a. Specific Objective: Determine the nature of biodegraded products of wheat straw and kenaf lignin by Cyathus stercoreus.

      Progress: Thirty percent of the lignin in wheat straw was degraded after 30 days fermentation with Cyathus stercoreus to $CO_2$, EtOH, MeOH, acetic and high molecular weight, water soluble, lignin-like polymers. Molecular weights of the soluble degradation polymers range from 15,000 to 20 X $10^6$ based on comparable GPC exclusion volumes of standard high molecular weight dextrans.

      Kenaf plants were grown in environmental chambers and fed $^{14}C$-phenylalanine to label the plant lignin to follow the degradation products of lignin in kenaf with Cyathus stercoreus.

   b. Specific Objective: Initiate new studies to determine the mechanism of lignin biodegradation by Phanerochaetae chrysosporium in liquid culture for possible chemical imitation.

      Progress: The literature dealing with lignin biodegradation by white-rot fungi and other microorganisms was reviewed in depth and several possible routes of investigation were identified. Suitable growth conditions for Phanerochaetae were developed. Preliminary results suggest a central role for an activated oxygen species in the initial steps of lignin biodegradation.

   c. Specific Objective: Develop non-destructive techniques and methodologies for qualitative and quantitative physico-chemical studies of insoluble plant components such as lignin both in vivo and in vitro.

      Progress: From the literature, it appeared that photoacoustic spectroscopy (PAS) could be a suitable, non-destructive technique for spectroscopic investigations of insoluble and/or opaque samples of plant material. The suitability of PAS for studies of lignin in vivo was confirmed by preliminary experiments at UOP Research, Des Plaines. A dual channel PAS system for NRRC was designed and the components purchased.

2. Basic Studies on Modification of Natural Polymers as Replacements for Petroleum-Derived Polymers (F. H. Otey)

   a. Specific Objective: Initiate new studies on methods and mechanisms to modify biopolymers to provide an expanded knowledge base for replacing petroleum-derived polymers with natural polymers.

      Progress: Work was directed primarily toward replacing petroleum-derived polymers with carbohydrate natural polymers for thermoplastic applications. Carbohydrate natural polymers are inherently water

sensitive, rigid, and brittle due to high crosslinked densities, molecular branching, and hydrogen bonding. For these reasons, such natural polymers must be combined chemically or physically with other products in order to produce flexible, water resistant composites for thermoplastic applications. Techniques were developed and applied to the synthesis of several benzyl ethers of starch having 0.05 to 3.0 benzyl per anhydroglucose units (AGU). Also, a series of starch esters were prepared including the benzoyl, octanoyl, and palmitoyl esters. Other modified starches obtained for the study included Amaizo 7420 and 80, Astro Gum 3020, Cato-14 Cationic, Amioca waxy maize, and SGP 502S (Super Slurper).

b. Specific Objective: Initiate a basic program to determine effects of new and novel physical and chemical modifications on the physical properties of biopolymers.

Progress: To determine the effects of various chemical modifications on natural polymers, techniques were devised for formulating these polymers into test specimens for stress-strain testing. This was achieved by combining the modified natural polymers with ethylene acrylic acid copolymer in a 40:60 ratio, respectively. The composites were then extruded into 1-in. wide specimens or extrusion blown into films and tested on an Instron. Preliminary results of these tests suggests that chemically adding very low levels (0.05 to 0.2 moles/AGU) of benzyl ether groups significantly improve the flexibility of natural polymer systems possibly through reduced hydrogen bonding. Higher derivatization reduced mechanical properties of the test specimens. Nine other modified starches were formulated and tested but the effects of these modifications were not clearly established.

c. Specific Objective: Study the aqueous dispersion characteristics of natural polymers and correlate the effects of various chemical additives on the flexibility of biopolymer molecules.

Alkali gelatinization and accompanying viscosity evaluations were completed on 17 different plant starch species and 12 plant flour species. The data, summarized in two rough draft manuscripts, identify the conditions needed to gelatinize these starches and flours and characterizes the resulting aqueous dispersions. Infrared absorption spectra were obtained on both available and synthesized polyols that are being evaluated as plasticizers. Both the gelatinization and polyol studies are providing basic information relating to the use of natural polymers in thermoplastic applications.

d. Specific Objective: Study graft polymerization onto the components of wheat straw.

Progress: Extraction of wheat straw with 10% sodium hydroxide, without prior removal of lignin, yielded a hemicellulose A fraction (lignin content about 11%) that was totally unreactive when attempts were made to initiate graft polymerizations of acrylonitrile with ceric ammonium nitrate. We showed that this lack of reactivity was due to the high percentage of lignin in the sample, since hemicellulose isolated from a delignified wheat straw was a reactive substrate in ceric-initiated graft polymerizations.

Although ceric ion was unsuitable as an initiator for graft polymerizations onto crude hemicellulose, polymerizations were successfully initiated with $Fe^{++}/H_2O_2$. The resulting hemicellulose-polyacrylonitrile graft copolymers were saponified with sodium hydroxide to yield thickening agents and water absorbents which had properties similar to the analogous starch-based products. A hemicellulose-poly(methyl acrylate) graft copolymer was also prepared using $Fe^{++}/H_2O_2$ initiation, and this polymer could be extrusion-processed into a continuous plastic.

e. Specific Objectives: Determine effects of new and novel physical and chemical modifications on the physical properties of lignin.

A preparation of the model compound of lignin, guaiacylglycerol-β-guaiacyl ether, is partially completed. This compound will be used to do basic studies on lignin modification reactions.

f. Specific Objective: Study the influence of crystallinity and hydrogen bonding in cellulose fibers on the graft polymerization of thermoplastics and on the properties of extruded graft copolymers.

Progress: The ceric-initiated graft polymerization of methyl acrylate onto bleached cellulose pulp was studied with cellulose samples that had been given different pretreatments to alter crystallinity and hydrogen bonding. Cellulose samples were subjected to high speed stirring (Valley beater) and were also treated with sodium hydroxide (mercerization) and with phosphoric acid before graft polymerization. Graft copolymers contained 50-55% grafted poly(methyl acrylate); however, unlike their starch analogs, the plastics formed from these materials by extrusion processing were all extremely brittle.

Treatment of a graft copolymer with 70% zinc chloride, in an attempt to reduce crystallinity, did not improve properties of the plastic, and mastication of the graft copolymer by cold rolling also gave a brittle product on extrusion. We were able to prepare cellulose-poly(methyl acrylate) plastics with greatly improved properties if a portion of the cellulose was removed from the graft copolymer by enzymatic hydrolysis prior to extrusion.

g. Specific Objective: Study chemical and physical treatments of starch and cereal grains with the objective to lower energy requirements of starch gelatinization, liquefaction, and saccharification.

Progress: A continuous enzymatic process for gelatinization, liquefaction, and saccharification of starch and cereal grain substrates has been developed and demonstrated as an efficient technique for converting these substrates to fermentable sugars.

The continuous process involved the sequential incorporation of the starch substrate, solutions of a thermostable alpha-amylase for gelatinization/liquefaction, and solutions of buffered glucoamylase into the continuous mixer (Model 50 Ko-Kneader, Baker Perkins, Inc.) under conditions conducive to rapid enzymatic hydrolyses. In the evaluation of numerous processing variables, optimization of the

relatively high-energy gelatinization/liquefaction step was achieved by using 50-60% substrate concentrations, 95°C, 0.4% alpha-amylase, and a residence period of 6 minutes. In addition to energy and labor saving advantages associated with a processing technique of this type, viscosity reductions, and formation of reducing groups were achieved more rapidly than in the laboratory under a variety of batch gelatinization/liquefaction conditions. However, rates and extents of glucose formed from well-liquefied substrates in both the continuous and laboratory procedures were alike.

h. Specific Objective: Initiate new studies to determine which of a wide variety of modifications to biopolymers impart significant soil-aggregate stabilizing activities.

Progress: Starch derivatives were compared with polyvinyl alcohol and with polyvinyl acetates by wet sieving techniques to obtain an indication of soil stabilizing potential. Certain benzyl ethers of both starch and hydroxyethylated (HE) starch exhibited stabilizing properties. HE starch with 9% benzyl content was the most effective derivative examined, stabilizing 63 and 96% of the soil to at least 10 min of water immersion with abrasion at dose levels of 0.04 g and 0.3 g per 100 g of dry soil, respectively.

Close contact has been maintained with Dr. W. C. Moldenhauer (ARS Soil Scientist, Purdue University) who has provided suggestions and test procedures and has expressed interest in evaluating the more promising products of this study.

i. Specific Objective: Examine the extrusion of carbohydrate-poly(methyl acrylate) graft copolymers and study the physical properties of the resulting plastics.

Progress: Ultimate tensile strength (UTS) of granular starch-g-poly(methyl acrylate) (S-g-PMA) was shown to rise as the starch content of the graft copolymer increases over the range 25-58%. The degree of increase depends on the strain rate so that UTS increases only from 2.2 to 2.6 $Kg/mm^2$ at 5 cm/min cross-head velocity but increases from 1.9 to 3.2 $Kg/mm^2$ at 50 cm/min. The UTS was unaffected by increasing molecular weight of the grafted poly(methyl acrylate) from 500,000-1,000,000. Changing reaction temperature from 27 to 40°C did not influence UTS values. Retention of low levels of homopolymer (less than 15%) reduced the UTS only about 3-5%. Excessive contact of the graft copolymer with the ceric initiator reagent reduces tensile strength somewhat and increases the amount of extractable homopolymer. Most damage by initiator reagent is caused by the ceric ions. Elongation of the tensile specimens and total energy to break was greatest for the S-g-PMA copolymers having the largest PMA contents. In contrast to previous tests, high shear milling of the S-g-PMA on the rubber rolls did not make it more rubbery and these samples were not amenable to blow molding.

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3. Basic Studies on Biopolymers for Improving Safety of Pesticides (F. H. Otey)

a. Specific Objective: Investigate metal-natural polymer complex systems for encapsulating pesticides and determine effects of these systems on the release rate of pesticides.

Progress: Improved conditions were obtained to encapsulate specific pesticides using starch-calcium adduct as an encapsulating agent. Performance improvements were recorded in greenhouse and field studies using encapsulated thiocarbamate and trifluralin herbicides in comparison with commercial formulations. Storage stability of encapsulated insecticide diazinon was found greatly improved upon encapsulation using this technique. It was also discovered that starch-calcium adducts can easily encase elastomers such as natural rubbers and latexes to provide powdered and crumb rubber.

Another technique was discovered to encapsulate water-insoluble pesticides, including emulsifiable concentrates and wettable powders. It consists of gelatinizing a slurry of pearl starch containing pesticide followed by boric acid treatment. Important advantages of this technique are high recovery of active ingredient to be encapsulated and the elimination of filtration step needed with the calcium and the xanthide procedures.

b. Specific Objective: Investigate the incorporation of modified natural polymers into spray applied tank mix systems as a potential approach to controlling pesticide release and reducing losses due to evaporation and decomposition.

Progress: Evaporation of 1% alkali starch-butylate pesticide dispersions at 0.5-5% starch concentrations yielded films retaining up to 80% of the butylate. Difficulties were encountered obtaining reproducible results due to variations in film homogeneity and dispersion stability during evaporation. Photomicrographs of film cross-sections showed many tiny pores similar to those observed in starch-based encapsulation systems. When such dispersions were evaporated to dryness in the presence of sand, about 45% of the butylate was retained after 1 day. In the absence of starch, retention of butylate in sand was less than 10% after the same period. Synthetic polymers such as polyvinyl alcohol did not perform as well as starch in this application.

c. Specific Objective: Initiate studies to evaluate reaction variables to maximize encapsulation of pesticides into biopolymer matrixes.

Progress: Reaction variables such as chemical ratios, oxidation or crosslinking chemicals, rate of mixing, and effect of sonification have been evaluated for the starch xanthide encapsulation technique to maximize encapsulation and alter rate of release. Preliminary work has been started on the rate of release studies starch-calcium and starch-borate encapsulation processes. Sonification of commercial emulsifiable concentrates in water before encapsulation in a starch matrix reduces the size of the pesticide pockets by a factor of 10 as shown by scanning electron microscopy. Rate of release of sonified

pesticide encapsulated products is more uniform and slower than unsonified products as shown by accelerated aging in humidity chamber studies.

d. Specific Objective: Continue cooperation with academia, industry, and government scientists by formulating and providing suitable encapsulated samples for greenhouse and field evaluation.

Progress: Nearly 50 samples of encapsulated pesticides were provided to 8 collaborators from academia, industry, and government. Comparative data were obtained on samples prepared by the xanthate, calcium adduct, and borate procedures versus commercial formulations. Samples were made in pound quantities in all of the procedures using a variable speed double planetary mixer obtained this past year. Samples of the newer borate encapsulation procedure were tested with promising results. Trifluralin and butylate were encapsulated by the xanthate-iron crosslinking procedure to give products passing 40 mesh for tank mix spray applications. Previous field trials with encapsulated trifluralin revealed that the rate of release of active ingredient is too slow for effective weed control. This deficiency was corrected by coating technical trifluralin onto the encapsulated granular particles.

e. Specific Objective: Conduct toxicological studies on encapsulated pesticide formulations that show greatest promise.

Progress: Since encapsulation greatly reduces rate of pesticide volatization, we believe that it may significantly improve worker safety associated with the storage, shipment, and application of pesticides. Procedures were developed with the cooperation of Dr. Ronald T. Riley, Research Pharmacologist (ARS, Athens, Georgia) who has agreed to conduct percutaneous permeability experiments using pig epidermis and $^{35}S$-parathion. The labeled pesticide was ordered and methods for encapsulating small amounts were developed.

f. Specific Objective: Initiate studies to formulate mathematical expressions for the absolute rate of dissolution and/or diffusion of particular natural-polymer encapsulated pesticide compositions to provide a basis for understanding the relationship between composition variables and release rate of the pesticide.

Progress: Studies of the kinetics of sustained release of trifluralin from starch xanthate-encapsulated formulations under laboratory conditions indicate that the rate-determining step is controlled initially by diffusion at the particle surface (boundary layer-control) and subsequently by diffusion in the polymer (matrix-control). These and other preliminary observations suggest that the basic principles and mathematical models employed in the design and evaluation of controlled-release systems for pharmaceuticals are applicable to encapsulated pesticides and the models, with some modification, are expected to provide the theoretical framework for a mechanistic interpretation of sustained release of pesticides from biopolymeric systems.

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4. New and Improved Technologies for the Fermentative Production of Chemicals from Biomass (R. J. Bothast)

   a. Specific Objective: Determine the extent of acrolein formation by lactobacilli.

      Progress: β-Hydroxypropionaldehyde is a precursor to acrolein which is an important intermediate for making acrylic acid and a variety of other useful industrial chemicals. Glycerol dehydrase was isolated from Lactobacillus sp. NRRL B-1720 and used to convert glycerol to β-hydroxypropionaldehyde in the presence of Vitamin $B_{12}$ coenzyme and $NH_4+$ ion. When saturated with substrate, each gram of soluble protein produced as much as 0.32 g of the aldehyde per hour at 25°C. However, enzyme activity was lost within 60 to 90 minutes of reaction initiation.

5. Recovery of Rubber from Guayule and Related Whole Plant Materials (G. E. Hamerstrand)

   a. Specific Objective: Develop standardized analytical methodology for determining latex content of guayule shrubs.

      Progress: A rapid analytical procedure for determining the quantity of rubber in guayule was developed. The gravimetric method is based on use of a high speed mixer-homogenizer to disintegrate the shrub in the presence of suitable solvents. Disruption of the cell walls by this technique makes the rubber readily accessible to solvent extraction. This precludes the use of the time-consuming soxhlet extractions previously used which reduces the time required for the analyses from approximately 80 hours to 3 hours.

   b. Specific Objective: Determine the efficacy of super critical fluids for extracting resins and rubber from guayule.

      Progress: The ability of super critical carbon dioxide to remove some of the hydrocarbons from guayule was demonstrated. However, the super critical extraction method was not pursued due to the urgency for developing pilot technology for the more conventional solvent extraction process.

   c. Specific Objective: Conduct pilot scale extraction studies for the recovery of rubber from guayule.

      Progress: The solvent extraction process developed on a laboratory scale at NRRC was evaluated to determine its applicability to existing pilot-scale equipment at the Center. Tentative process flow patterns were established and the major items of equipment required for the proposed pilot-scale process have been identified. Aspects of the solvent process not adequately covered in the laboratory studies have also been examined and pilot technology developed. This includes techniques for leaf removal, chopping, and grinding of whole plant material.

6. Hydrolysis of Hemicelluloses by Enzymes from Anaerobic Bacteria (Cooperative Agreement - Virginia Polytechnic Institute and State University)

   a. Specific Objective: Develop new biochemical processes using enzymes from anaerobic bacteria to hydrolyze plant hemicelluloses to fermentable sugars for the production of chemical feedstocks.

      Progress: Experimental procedures were jointly planned and residue samples were provided to the cooperator. A specific anaerobic strain was selected for the study and analytical techniques were developed to determine the nature and extent of polysaccharide degradation.

7. Hydrocarbon-Producing Plants as Potential Multi-Use Crops (M. E. Carr)

   See Northern Agricultural Energy Center, A.1.

8. Increased Energy Efficiency of Substrate Preparation for Alcohol Fermentations (R. J. Bothast)

See Northern Agricultural Energy Center, A.2.

9. Innovative Fermentation Technology for Alcohol Production (R. J. Bothast)

See Northern Agricultural Energy Center, A.3.

## D. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

1. Principles Underlying Design of Food Blends for the Export Market (R. A. Anderson)

a. Specific Objective: Continue development and evaluation of new cereal food products with improved nutritional quality.

Progress: The testing of 1 and 2% added tricalcium phosphate (TCP) for compatability with various PL-480 commodities is continuing. Efficacy in inhibiting insect infestation was demonstrated in bulgur, soy-fortified bulgur, soy-fortified rolled oats, soy-fortified sorghum grits, soy-fortified bread flour, and soy-fortified corn meal. The inclusion of 1 part soybean oil with each 2 parts TCP was necessary to reduce dusting and provide homogenous non-separating mixtures. Stability studies are in progress to monitor the effect of added soybean oil on peroxide values, fat acidity, flavor and functional properties. Stability was satisfactory after 3 months' storage at 37°C.

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BOOKWALTER, G. N. Meeting Nutritional Objectives with Soya Proteins. J. Am. Oil Chem. Soc. 58 (1981):528-529.

BOOKWALTER, G. N. Labeling and Compliance Assurance of Soya Protein Foods. J. Am. Oil Chem. Soc. 58 (1981):529-530.

BOOKWALTER, G. N., T. P. SHUKLA, AND W. F. KWOLEK. Salmonellae Destruction in a Low Moisture Food with Microwave Power. Proceedings 16th Annual Symposium, International Microwave Power Institute, pp. 91-92. 1981.

BOOKWALTER, G. N. AND W. F. KWOLEK. Predicting Protein Quality of Corn-Soy-Milk Blends after Nonenzymatic Browning. J. Food Sci. 46 (1981):711-715.

TRAVER, L. E., G. N. BOOKWALTER, AND W. F. KWOLEK. A Computer-Based Graphical Method for Evaluating Protein Quality of Food Blends Relative to Cost. Food Technol. 35 (1981):72-78.

Other Reports:

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BOOKWALTER, G. N., T. P. SHUKLA, AND W. F. KWOLEK. Salmonellae Destruction in a Low-Moisture Food with Microwave Power. International Microwave Power and Institute Symposium, Microwaves in the Food Industry, Toronto, Canada, June 1981.

# CEREAL SCIENCE AND FOODS LABORATORY

G. E. Inglett, Chief

Research Leaders: E. B. Bagley, F. R. Dintzis, and J. S. Wall

## A. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

### 1. Interactions of Food Carbohydrates (E. B. Bagley)

a. Specific Objective: To investigate newly discovered factors affecting the gelatinization and retrogradation of starch as related to food quality in cereal food processing.

Progress: Hydrocolloid gums were found to prevent retrogradation and gel formation of corn starch when hot dispersions were cooled to room temperature. This is significant in starch based foods since the inhibition of retrogradation and gel formation can improve properties such as storage stability and organoleptic acceptability. To understand the mechanism of the action of these hydrocolloids in starch dispersions, experiments were undertaken to establish quantitatively the effects of starch granule swelling alone on dispersion properties. Corn and wheat starch dispersions in water were heated to controlled temperatures in the starch gelatinization range (65-80 for corn; 60-75 for wheat) and viscosities were determined at both 60°C and at room temperature. As the dispersions cooked qualitative changes in the flow curves (viscosity-shear rate plots) were seen. Thus, dilatancy (increase of viscosity with shear rate) was observed when the granules were relatively rigid and close packed. This occurred at short cook times and/or low cook temperatures. As the granules swelled and softened, the dispersions showed shear thinning effects, the viscosity decreasing with increasing shear rate. When swelling and softening was complete, independent of the cook temperature, viscosity was found to be dependent only on the volume fraction of the swollen starch granules in the dispersion. Scanning electron microscopy studies served to correlate granule morphology with the viscosity behavior. In the temperature ranges examined, exudate from the swollen granules was unimportant in determining viscosities, but can be expected to be significant at higher cook temperatures.

Publications:

BAGLEY, E. B. AND D. D. CHRISTIANSON. Swelling Capacity of Wheat Starch and its Relationship to Suspension Viscosity--Effect of Cooking Time and Temperature and of Concentration. Accepted for Journal of Texture Studies.

CHRISTIANSON, D. D. AND E. B. BAGLEY. Viscosities of Dispersions of Swollen Corn Starch Granules. For Cereal Chem., in preparation.

CHRISTIANSON, D. D., A. R. LOFFREDO, F. R. BAKER, AND E. B. BAGLEY. Correlation of Microscopic Structure of Corn Starch Granules with Rheological Properties of Cooked Pastes, in preparation.

CHRISTIANSON, D. D., J. E. HODGE, D. OSBORNE, AND R. W. DETROY. Gelatinization of Wheat Starch as Modified by Xanthan Gum, Guar Gum, and Cellulose Gum. Cereal Chem. 58(6) (1981):513-517.

CHRISTIANSON, D. D. Hydrocolloid Interactions with Starches. Book Chapter in Proceedings of Basic Symposium on Food Carbohydrates. Accepted for publication, 1982.

Other Reports:

BAGLEY, E. B. Flow of Starch Dispersions. Invited presentation at Seminar at General Foods Central Research Laboratory, Larrytown, NY, September 30, 1981.

BAGLEY, E. B. Rheology of Dispersions of Deformable Particles. Invited presentation at Seminar, Material Science Group, Department of Mining and Metallurgical Engineering, University of Illinois, Champaign/Urbana, Illinois, November 13, 1981.

BAGLEY, E. B. Rheology of Dispersions and Gels. Invited presentation, Kent State University, Department of Chemistry, Kent, Ohio, June 17, 1981.

BAGLEY, E. B. Food Carbohydrate Interactions: Rheological Properties. Review of NRRC Cereal Food Science Research, Peoria, Illinois, June 29, 1981.

BAGLEY, E. B. Rheology of Doughs. Twenty-Second Annual Corn Dry Milling Conference, NRRC, Peoria, Illinois, June 3, 1981.

BAGLEY, E. B. Thirty hours of lectures on Food Rheology in Department of Food Science, University of Illinois, Champaign/ Urbana, Illinois, June/July 1981.

CHRISTIANSON, D. D. Hydrocolloid Interactions with Starches. Presented at the IFT Basic Symposium on Food Carbohydrates, Atlanta, Georgia, April 1981.

2. Corn Starches--Physical Characteristics and Biological Digestibilities (F. R. Dintzis)

   a. Specific Objective: Adapt differential scanning calorimetry (DSC) techniques to single and double corn mutants.

      Progress: The use of differential scanning calorimetry (DSC) of amylose-lysolecithin complexes for quantitative determination of amylose content of single-kernel corn samples was not implemented because of many uncertainties in the technique. An alternative method of amylose determination has been developed; the procedure utilizes

the well-known formation of the blue amylose-iodine complex. The new method is unique, however, in that no KI is required to form the $I_3^-$ ion necessary for initiation of the complex formation. It was discovered that $I_3^-$ is formed upon dissolution of iodine in DMSO. Thus, by dissolving starch samples in DMSO containing iodine, the amylose-iodine complex can be formed spontaneously merely by dilution with water. Amylose is determined by measuring the absorbance of the complex. The method works equally well on pure starch samples, or ground whole corn or ground endosperm. The method has been applied to single-kernel samples of maize varieties containing known amounts of amylose from 25 to 80%, and the expected variation between kernels in each variety has been evaluated.

Development of techniques for evaluation of gelatinization behavior by DSC of single-kernel samples is still in progress. Use of dry-ground samples has been found to be unsatisfactory because of interference of protein in the sample. Small-scale methods for removal of protein are being investigated.

Publication:

KNUTSON, C., J. CLUSKEY, AND F. R. DINTZIS. Properties of Amylose Iodine Complexes Prepared in the Presence of Excess Iodine. Carbohydrate Research, in press.

3. Fundamental Studies on Separation of Starch, Protein, and Lipid of Corn (E. B. Bagley)

See Biomaterials Conversion Laboratory, B.2.

4. Interactions of Dietary Fibers from Cereal Products with Mutagens in Digested Food (F. R. Dintzis)

a. Specific Objective: To determine the presence of mutagens in human fecal samples from volunteers being fed regulated diets containing dietary fiber.

Progress: Fecal samples supplied by the Human Nutrition Laboratory, Grand Forks, ND, were screened for mutagens by the Ames Test in cooperation with the Environmental Laboratory at the University of Illinois, Champaign-Urbana. In the last group of 6 samples, there were 3 negative, one toxic, and two suspicious enough to warrant further investigation. Model food systems were treated under realistic conditions to determine if cooking parameters would lead to development of mutagens. Of 36 samples screened by the Ames Test, 13 samples indicated further work would be needed to verify if mutagens were generated.

The number of personnel required to thoroughly support these efforts has been greater than is deemed justifiable with limited resources. Therefore, this project is being terminated.

Under this project, some efforts have been spent on an investigation of improved methodology to characterize dietary fiber. These efforts have led to a publication.

Publication:

LEHRFELD, J. Differential Gas-Liquid Chromatography Method For Determination of Uronic Acids in Carbohydrate Mixtures. Anal. Biochem. 111 (1981):410-418.

5. Methods of Analysis to Facilitate Genetic Improvements of Cereal Grain Protein (J. S. Wall)

a. Specific Objective: Determine $NH_2$-terminal amino acid sequences of endosperm storage proteins from different cereals.

Progress: Prolamins have been isolated from samples of milled millet and rice grains, and their $NH_2$-terminal amino acid sequences have been determined by automated Edman degradation. The data obtained for millet prolamins are primarily a single sequence, although the sample is heterogeneous; these are the first sequence data available for millet proteins. Rice prolamins are not cleaved by the Edman degradation, suggesting a blocked $NH_2$-terminal residue. Millet prolamin sequences and those previously obtained for prolamins from other genera exhibit marked intra-genus homology, demonstrating that in all cereals they arise from translation of a group of closely related genes which arose through duplication and mutation from single ancestral genes. Inter-generic homology of prolamins is also apparent among cereals, showing that prolamin sequences can demonstrate, clarify, and predict genetic interrelationships of cereal grains.

Preliminary $NH_2$-terminal amino acid sequence results have been obtained for isolated secalins (rye prolamins). C-secalin has been shown to be homologous to C-hordein from barley and to omega-gliadin from wheat. B-secalin has been shown to be homologous to wheat gamma gliadins.

The $NH_2$-terminal amino acid sequence has been determined for alcohol-soluble reduced glutelin subunits of grain sorghum. The results demonstrate close homology, if not identity, to kafirins, the prolamins from sorghum, in agreement with previous studies in which these two protein fractions were isolated and compared by amino acid analyses and electrophoresis. In contrast, alcohol-soluble glutelins from wheat or from corn exhibit major differences in $NH_2$-terminal amino acid sequence to the major prolamin fractions. Some investigators would like to combine prolamins and alcohol-soluble glutelins into a single fraction, soluble in alcohol under reducing conditions, but such a definition is clearly inadvisable, since it combines significantly different fractions in some cereals.

b. Specific Objective: Obtain further detailed amino acid sequence information for isolated corn endosperm protein.

Progress: Prolamine fractions were isolated from endosperm meal of dry milled inbred W64A corn by successive selective extraction using the Landry-Moureaux procedure in combination with the Paulis-Wall procedure. Comparison of proteins of the two procedures showed similar proteins. Zein was fractionated with 75% ethanol by gel filtration chromatography using a chemically prepared organic gel from sephadex G-100 (LH100). The 22,000 MW subunit fraction was isolated and is now being analyzed for its $NH_2$-terminal amino acid sequence. New innovative gel electrophoretic techniques using Sephadex G-200 superfine as a gel and formamide as solvent were tried in an effort to isolate zein polypeptides by electrophoresis combinations at pH 3.5 and isoelectric focusing (IEF) at pH 6-8. Thin-layer polyacrylamide gel electrophoresis (PAGE) using 8M urea aluminum lactate pH 3.5 and sodium dodecyl sulfate (SDS) were developed as analytical methods for detecting charge heterogeneity for micro-quantities of isolated zein polypeptides.

A number of individual zein polypeptides have been isolated by preparative ion-exchange chromatographic and electrophoretic methods, and their $NH_2$-terminal amino acid sequences have been determined as an initial step in determining an entire zein sequence and demonstrating the basis for variability between zeins. Single amino acid sequences result for each preparation, demonstrating their homogeneity and suitability for further degradations and analyses. Of particular interest is the fact that two isolated zeins having the same apparent molecular weight, 22,000 (that is, the lower of the two major molecular weight species), have significantly different $NH_2$-terminal sequences, namely, $NH_2$-T-I-F-P-Q-C-S-Q-A-P-I-A-S-L-L-P-P-Y-L-P- and $NH_2$-F-I-I-P-Q-C-S-L-A-P-S-A-I-I--Q-F-L-P-. The sum of these sequences approximates that for whole zein, these results demonstrate that the previous supposition that the two major sequences present in total zein represent the two major molecular weight species is erroneous.

A number of specific chemical and enzymatic cleavage methods have been tested for their ability to produce zein peptides suitable for further characterization and sequence analysis as part of the determination of a total zein sequence. Of the methods initially tested, trypsin cleavage looks most promising, and produces peptides of 4,000 to 6,000 daltons; some of these peptides have been purified by preparative gel filtration and electrophoretic methods, and are now being characterized and subjected to amino acid sequence analysis.

A methionine-containing protein has also been isolated from reduced corn glutelin and has been partially characterized. Its apparent molecular weight is 15,000-16,000, and its $NH_2$-terminal amino acid sequence is $NH_2$-M-Q-M-P-G-P-F-A-G-L-Q-G-L-Y-G-A-G-A-G-Q-. The apparent high methionine content of this protein gives it potential nutritional significance.

c. Specific Objective: Apply improved electrophoretic and chromatographic methods to analysis of proteins to analyze their genetic control, to serve as a basis for breeding wheat varieties having better quality, and to devise rapid quality tests.

Progress: High-performance gel filtration chromatography, employing columns having three different molecular weight separation ranges, has been extensively tested with cereal proteins. Neutral phosphate buffers containing the anionic detergent sodium dodecyl sulfate have been found most suitable for the protein solubilizing agent and eluent, reducing agents can be added or omitted, depending on whether native proteins or their constituent polypeptide subunits are to be analyzed. Column effluents are monitored either at 280 nm or at 210 nm; the use of 210 nm leads to enhanced sensitivity, and the ability to detect proteins' peptide bonds, rather than only aromatic amino acids. A new computer method has been devised which permits accurate quantitation of results and determination of molecular weights. These methods are extremely reproducible, and molecular weights determined agree closely with other methods. In addition, approximately 50 analyses per day are now possible, compared to 3 or 4 per week by traditional open-column gel filtration methods.

Using these methods, a large number of isolated cereal proteins and protein classes have been examined to determine their molecular weights, compare samples, demonstrate disulfide bonding, demonstrate conformational changes, and show homogeneity or heterogeneity. In addition, methods have been adapted which permit isolation of total proteins (reduced or non-reduced) or glutelins from single wheat kernels; using these methods, a large number of bread and durum wheats and their aneuploids have been examined. Considerable variability in the elution profiles is evident, demonstrating the possibility of varietal identification by high-performance gel filtration chromatography. In addition, the methods permit demonstration of varietal purity, and selection in breeding programs on the basis of variability. A probable high inverse correlation has been noted between the amount of high-molecular weight protein observed in chromatograms and the mixing times, strengths, or qualities of a large group of well-characterized hexaploid wheats, suggesting that a rapid bread wheat quality test may be possible by high-performance gel filtration chromatography.

In an initial application of these methods, a number of normal (vitreous) and yellow-berry (opaque) selections from several Triticale lines were examined. Reduced total protein extracts of yellow-berry kernels contained significantly more high molecular weight glutelin subunits than did the normal kernels, possibly leading to the differences in endosperm vitreosity.

Improved methods for polyacrylamide gel electrophoresis (PAGE) were also explored. Several modifications of PAGE in acid buffers were incorporated into our methodology. These procedures gave sharper resolution and reduced time of operation and cost of materials. Vertical electrophoresis in SDS medium was shown to result in better resolution of proteins of similar molecular weight. Application of these methods to analysis of varieties established differences in protein composition, the importance of which is being evaluated.

d. Specific Objective: To confirm and extend knowledge on sites of synthesis and deposition of wheat endosperm proteins.

Progress: Various techniques of dehydrating and infiltrating the immature wheat kernel were investigated in order to fix the protein bodies and other protein components without crosslinking them, so they can be extracted by various solvents normally used to separate the protein fractions. Other techniques such as cutting and staining were improved. While improvements have been made, the extraction of proteins from the protein bodies and other structures with specific solvents have not resulted in clear cut differences. Whether the difficulty is due to imperfect methods or whether the proteins are normally modified by our usual extraction procedures needs to be established.

e. Specific Objective: To study the relationship between variation in genetic background of corn inbreds and their zein component inbreds and to possibly locate genes responsible for coding specific zein proteins.

Progress: A 2-dimensional polyacrylamide gel electrophoresis system using aluminum lactate buffer -8M urea, pH 3.5, in the first direction followed by isoelectric focusing in 8M urea (pH 6-8) was developed to improve separation of zein polypeptides to help establish the composition of zein extracts in more detail. Isoelectric focusing in polyacrylamide gels was used to compare the zeins in extracts from a number of inbred lines of corn and their hybrids. The patterns were recorded on a densitometer and the data was analyzed by computer. Variations in zein patterns among the inbreds were observed. The patterns of the hybrids did not always conform to that expected from the patterns of the parental inbreds.

f. Specific Objective: Determine the molecular conformation of corn alcohol soluble reduced glutelin fraction (ASG) by optical rotatory dispersion (O.R.D.).

Progress: The molecular conformations of zein, ASG, water-soluble ASG, and water-insoluble ASG were determined in 70% ethanol-0.5% NaOAc-0.1M-β-mercaptoethanol by O.R.D. and circular dichroism measurements. The α-helical contents of ASG, water-insoluble ASG and water-soluble ASG are 30, 28, and 17%, respectively. Zein has 38% α-helix in the reducing solvent and 41% α-helix in 70% ethanol-0.5% NaOAc. Disulfide bonds are not important in zein to maintain α-helical structure. The lower α-helical content of water-soluble ASG is consistent with its higher proline content compared with ASG and water-insoluble ASG.

g. Specific Objective: Determine the effect of germination of triticale on protein fractions and amino acid composition.

Progress: Triticale, a cross between wheat and rye, was germinated for 1 to 8 days. Lysine content of germinated triticale increased after 8 days from 3.5 to 5.9 g per 16 g nitrogen. A large increase in water-soluble nitrogen (rich in lysine) and a large decrease in prolamin (low in lysine) accompanied sprouting. The percent protein in triticale germinated for 3 days or more is greater than in the initial grain as a result of dry matter loss in the grain during germination, but the absolute amount of protein per kernel is not increased.

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WU, Y. V. Food-Related Research at the Northern Regional Research Center. ACFSTA Newsletter 3(3) (1981):4-6.

WU, Y. V. and A. C. STRINGFELLOW. Protein Concentrate from Air Classification of Flour and Horny Endosperm from High-Lysine Sorghum. J. Food Sci. 46 (1980):304-305.

Other Reports:

BIETZ, J. A. and L. A. COBB. High-Speed Gel Filtration Chromatography of Cereal Proteins. Presented at the AACC 66th Annual Meeting, Denver, Colorado, October 25-29, 1981. Cereal Foods World 26(9) (1981):484-485.

BIETZ, J. A., A. ESEN, J. W. PAULIS, and J. S. WALL. Amino Acid Sequence Investigations of Isolated Corn Endosperm Proteins. Presented at the AACC 66th Annual Meeting, Denver, Colorado, October 25-29, 1981. Cereal Foods World 26(9) (1981):500.

CHARBONNIER, L. and J. A. BIETZ. N$_t$ Sequences of C and B Secalins. Presented in poster session S5, "Rye prolamins: extractability, separation and characterization" at the International Symposium Seed Proteins, Versailles, France, September 22-24, 1981.

LANDRY, J. and J. W. PAULIS. Isolation and Characterization of Three Main Groups of Alcohol-Soluble Proteins from Maize Grain. Presented at the AACC 66th Annual Meeting, Denver, CO, October 25-29, 1981.

6. Expoxides from Lipid Hydroperoxides and Their Interactions in Cereal and Oilseed Foods (H. W. Gardner)

a. Specific Objective: Test fatty endoperoxides for toxicity and/or mutagenicity.

Progress: An isomeric fatty cyclic peroxide was isolated from a mixture obtained by the free radical decomposition of the 13-hydroperoxide of linolenic acid. This compound and the 13-hydroperoxide of linoleic acid were assessed for mutagenicity by the Ames Test. The weak mutagenicity of the hydroperoxide was confirmed.

b. Specific Objective: Volatile formation from "secondary products" of lipid oxidation.

Progress: Two diastereomers of methyl trans-12,13-epoxy-9-hydroperoxy-trans-10-octadecenoate were isolated for assessment of volatile production from a secondary product of lipid hydroperoxide decomposition. The volatiles as assessed by GC-MS were very similar to the volatiles obtained from decomposition of methyl 9-hydroperoxy-trans-10,cis-12-octadecadienoate, except the volatiles obtained from the epoxyhydroperoxyene fatty ester lacked 2,4-decadienals. Instead the isomeric epoxyhydroperoxyene fatty esters yielded a small to moderate amount of 4,5-epoxy-2-decenal. An unexpected result of the separation of secondary oxidation products was the isolation of methyl trans-12,13-epoxy-11-hydroperoxy-cis-9-octadecenoate. The existence of this compound has been implied but not shown directly.

c. Specific Objective: Explore novel ways of degrading hydroperoxides to obtain new information on mechanisms of rancidity development.

Progress: It was discovered that acids efficiently catalyze the decomposition of linoleic acid hydroperoxide. The decomposition occurs even in the presence of weak organic acids, like acetic; however, the catalysis with organic acids is much slower than with strong mineral acids. The decomposition of hydroperoxides with acetic acid may explain why pickled foods ordinarily do not become rancid. With all acids tested, linoleic acid hydroperoxide decomposed into fatty epoxides and their solvolysis products. The log k (k=rate constant) plotted a linear function with the Hammett Acidity Function.

Publications:

GARDNER, H. W. AND P. A. JURSINIC. Degradation of Linoleic Acid Hydroperoxides by a Cysteine-$FeCl_3$ Catalyst as a Model for Similar Biochemical Reactions. I. Study of Oxygen Requirement, Catalyst and Effect of pH. Biochim. Biophys. Acta 665 (1981):100-112.

GARDNER, H. W. AND R. KLEIMAN. Degradation of Linoleic Acid Hydroperoxides by a Cysteine-$FeCl_3$ Catalyst as a Model for Similar Biochemical Reactions. II. Specificity in Formation of Fatty Acid Epoxides. Biochim. Biophys. Acta 665 (1981):113-125.

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7. Molecular Structure of Maillard-Type Browning Reaction Products (H. B. Sinclair)

a. Specific Objective: Conduct browning reactions under cereal food processing conditions of heat treatment on isomaltol (2-acetyl-3-hydroxyfuran), lactose and maltose with secondary amines, and primary and secondary amino acids. Isolate browning reaction products and determine molecular composition of the water-soluble compounds so that knowledge will be obtained on the safety and affect on food quality.

Progress: Lactose and maltose have been reacted with primary and secondary amino acids. Lactose produces galatoctosylisomaltol and maltose produces glucosylisomaltol with secondary amino acids. Yields were 6 and 7% respectively. Primary amino acids gave yields less than 3%. This demonstrates that the free amino acids and amino functional groups of peptides and proteins react with maltose and lactose under conditions similar to those encountered during the baking of bread to form derivatives of isomaltol.

b. Specific Objective: Devise a better method for preparing 1-deoxy-1-amino-2-ketoses (Amadori compounds) to promote studies of nonenzymatic browning decompositions that reduce the nutritional values of processed foods.

Synthesize 1-deoxy-1-halogeno maltuloses having the reducing end stabilized in the furanose or pyranose form. These compounds will condense with amino groups of amino acids to form Amadori compounds that will serve as model compounds for studying nonenzymatic browning reactions.

Progress: A new approach to preparing the Amadori compounds was investigated. Glucitol (sorbitol) was converted in three steps to 1-tosyl-2,4-O-benzylidene-5,6-anhydroglucitol. The literature reports that a mild base treatment of this compound opens the 5,6-anhydro ring. However, this proves incorrect; what happens is that the displacement of the 1-toluene-sulfonate group by the 3-hydroxyl group occurs and a 4-membered ring is formed. Since the 1-tosyl group was to be the point of entry for the 1-amino group in 1-amino-2-ketose, the approach cannot be varied to circumvent this problem. Under this project work was completed on a study of glycols with different substituents attached and to study the stereochemical relationship of the glycols to sweetening power. Specifically, the various methanesulfonate (mesyl) esters of methyl α-D-glucopyranoside were to be prepared and displaced with different substituents. The displacement with base did not result in the expected products and the study was completed as follows:

Action of base on the di-, tri-, and tetra-O-mesyl esters of methyl α-D-glucopyranoside gave a series of methyl anhydro-O-mesyl hexoxides which further base treatment transformed in methyl anhydro-, dianhydro- or (various) anhydro-O-mesyl hexosides and one unusual olefinic hexoside. Two major points were discovered in this study: (1) the internal displacement preference of mesyl groups varies from the external preference $C_4>C_6>C_2$ (int.) vs. $C_6>C_4>C_2$ (ext.) and (2) a specific reaction sequence from among four reaction must be followed to account for the end products and a definite order of preference for these four reactions was determined.

Some progress has been made toward the synthesis of 1-deoxy-1-halogeno maltuloses.

Publications:

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VAN CLEVE, J. W. Synthesis of β-D-erythrofuranosyl β-D-erythro-Furanoside. In preparation for Carbohydrate Research.

Other Reports:

SINCLAIR, H. B. Products from the Base Treatment of the Tri-O and Tetra-O-methanesulfonyl Esters of Methyl α-D-glucopyranoside. Presented at the 181st American Chemical Society National Meeting, Atlanta, Georgia, March 29-April 3, 1981.

SINCLAIR, H. B. Chemistry of Browning and its Significance for Nutrient Availability. Presented at Gordon Research Conference on Nutrition, New London, NH, July 27-31, 1981.

8. Isolation, Purification, and Characterization of Corn, Alcohol-Soluble Proteins (Cooperative Agreement - Virginia Polytechnic Institute and State University)

Zein was fractionated into its individual polypeptide components by first subjecting it to chromatography on phosphocellulose (PC) columns and then using preparative isoelectric focusing in polyacrylamide gels to resolve components of the PC separations. A purified 22,000 mol. wt. zein polypeptide thus obtained was reduced and alkylated with vinyl pyridine and subjected to tryptic digestion in 50% dimethyl formamide. The resulting fragments were separated by ion exchange chromatography and preparative isoelectric focusing. Based on electrophoresis in sodium dodecyl sulfate media 2 different size classes of fragments were obtained, one with mol. wt. 6000 and another with mol. wt. 3500-4100. Purified zeins were also cleaved by cyangen bromide at methionine sites and the peptides separated. Both tryptic and cyanogen bromide cleaved peptides were submitted to J. Bietz of NRRC for amino acid sequence analysis.

Publication:

ESEN, A., J. A. BIETZ, J. W. PAULIS, AND J. S. WALL. Fractionation of Alcohol-Soluble Reduced Corn Glutelins on Phosphocellulose and Partial Characterization of Two Proline-Rich Fractions. Cereal Chem. 58 (1981):534-537.

## B. BIOMATERIALS SCIENCE

1. Enzymatic Conversion of Cellulose to Sugars for Alcohol Fermentations (F. R. Dintzis)

   a. Specific Objective: Characterize factors that limit efficiency of enzymatic conversion of cellulosics by "complete" cellulase systems to sugars for alcohol fermentation.

      Progress: A factor, now termed the "disruption factor," is being routinely isolated from a commercial preparation of the enzyme complex of Trichoderma reesei. Microscopic evidence shows the effect of this factor is to cause filter paper fibers to split off many small fibrils from the main fiber, or to cause sort of an unraveling effect. It is now established that ferric iron is necessary for effective disruption and that this disruptive effect can occur without any detectable hydrolytic activity upon the alpha cellulose substrate. It also has been established that prior chemical treatment of the alpha cellulose fiber greatly affects the disruptive action.

2. Energy-Saving Methods for Recovery of Usable Protein from Alcohol or Methane Fermentation Media (J. S. Wall)

See Northern Agricultural Energy Center, A.4.

## C. CHEMICAL RESIDUES AND ADDITIVES IN FOOD AND FEED

1. Effect of Environmental Contaminants on Cereal Foods and Feeds (W. J. Garcia)

   a. Specific Objective: Prepare corn food products intrinsically labeled with zinc-65 for subsequent use in determining the bioavailability of zinc in humans from corn foods primarily of endosperm origin.

      Progress: A defatted endosperm-hull fraction of whole kernel corn, derived from corn plants intrinsically labeled with radioactive zinc-65 via nutrient solutions, was incorporated with nonradioactive ground corn grits to eventually prepare a labeled food product, which was nonflaked, nontoasted, and primarily of endosperm origin. A second labeled food product was also prepared using identical quantities of intrinsically labeled endosperm-hull flour and corn grits; however, these combined materials were further processed into toasted corn flakes. Likewise, counterpart nonradioactive toasted corn flakes and nonflaked, nontoasted food products were also prepared. All four prepared products are currently being evaluated for bioavailability of zinc in human subjects jointly by the Human Nutrition Research Center and the University of North Dakota, both at Grand Forks. The relative abundance of nonradioactive zinc and zinc-65 present in the food products, at the start of the feeding studies and determined respectively by atomic absorption and gamma ray spectrometry, was found to be approximately 3 million times greater for the nonradioactive zinc.

      The subsequent bioavailability feeding studies will assess the absorption as well as the retention of zinc by humans. In addition, the specific effects of toasting and nontoasting of these prepared food products on the absorption of zinc will also be determined.

   b. Specific Objective: Prepare a defatted corn germ flour intrinsically labeled with zinc-65 to study human absorption of zinc in products where the labeled germ flour is to be used as a partial wheat flour replacement.

      Progress: A formulated corn germ flour to contain a fraction of intrinsically labeled corn germ was prepared from both nonradioactive and labeled corn germ flours. As the germ fraction of corn, the nonradioactive zinc content of the flour is considerably higher than in endosperm fractions of corn. New information on the availability of zinc from germ fractions will be derived from the subsequent feeding studies to be conducted at the Human Nutrition Research Center. The formulated germ flour will be used at a 20% replacement of wheat flour in preparing corn bread and muffins with an associated activity of 0.1μCi $^{65}Zn$ per serving at each of six specified feeding dates occurring over a 4-month period.

Publications:

GARCIA, W. J., C. W. BLESSIN, G. E. INGLETT, W. F. KWOLEK, J. N. CARLISLE, L. N. HUGHES, AND J. F. MEISTER. Metal Accumulation and Crop Yield for a Variety of Edible Crops Grown in Diverse Soil Media Amended with Sewage Sludge. Environ. Sci. Technol. 15 (1981):793-804.

CARLSON, K. D., R. L. CUNNINGHAM, W. J. GARCIA, M. O. BAGBY, AND W. F. KWOLEK. Performance and Trace Metal Content of Crambe and Kenaf Grown on Sewage-Treated Stripmine Land. Submitted to Environmental Pollution.

Other Reports:

GARCIA, W. J. Metal Translocation to Food Crops From Diverse Soils Amended with Sludge. Invited Presentation at Second Annual Illinois Water Pollution Control Association Meeting, Kankakee, IL, May 19-21, 1981.

GARCIA, W. J., A. J. PEPLINSKI, R. A. ANDERSON, AND G. E. INGLETT. Zinc-65 Intrinsically Labeled Corn Fraction Incorporated into Corn Food Products for Zinc Bioavailability Studies. Presented at the 66th Annual AACC Meeting, Denver, CO, October 25-29, 1981.

## D. FOOD COMPOSITION AND IMPROVEMENT

1. Action of Human Digestive System upon Cereal Grain Fiber Sources and Related Foods (F. R. Dintzis)

   a. Specific Objective: To examine effects of passage through the human digestive system upon wheat brans, dry milled corn bran, and soy hulls.

      Progress: Comparison of neutral sugar components of these dietary fiber sources demonstrates that xylose containing polymers are most susceptible to degradation in the human digestive system compared to arabinose or cellulose containing plant cell wall material. An effect of differential digestion as a function of particle size also is appearing. Thus, smaller particles have composition ratios different from those of the larger particles.

   b. Specific Objective: To measure the uronic acid content in dietary fiber materials.

      Progress: Attempts to use gas chromatography to quantitate the uronic acid content of dietary fiber material have uncovered serious questions regarding the hydrolysis of plant tissues to their component sugars using sulfuric acid. These questions involve interactions of uronic acids with sugars and with acid during the hydrolysis step. Continued work to resolve such questions is necessary to assure that valid results are obtained in dietary fiber analysis.

   c. Specific Objective: To examine factors influencing binding properties of cereal brans.

Progress: Experiments to date show that for wheat brans examined at pH ~1.5-2.0 at 37.5°C, in vitro, the binding of ferric iron at low concentration, e.g., 50 mg/L, is about equally divided between pericarp and those substances, such as solubles and endosperm, that slough off the bran in aqueous solution. However, at higher concentrations, of Fe, e.g., 400 mg/L, the pericarp binds only about 5% of the iron that is bound by other bran components. The binding of ferric iron to wheat bran is thus a function of iron concentration and binding is distributed between pericarp tissue and materials that are separated easily from pericarp tissue.

Publications:

DINTZIS, F. R. AND C. C. HARRIS. Starch Determination in Some Dietary Fiber Sources. Cereal Chem. 58 (1981):467-470.

GRAF, E. AND F. R. DINTZIS. High Performance Liquid Chromatography Method for the Determination of Phytate. Anal. Biochem. In press.

# FERMENTATION LABORATORY

C. W. Hesseltine, Chief

Research Leaders: R. W. Detroy, M. D. Grove, C. P. Kurtzman, O. L. Shotwell, and M. E. Slodki

## A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

### 1. Polysaccharides in Specific Associations of Nitrogen-Fixing Microbes with Plants (M. E. Slodki)

a. Specific Objective: Search for factors involved in preferential nodulation of soybean varieties by strains of Rhizobium japonicum.

Progress: Difficulties with the notion that Rhizobium capsular polysaccharide interacts with root-hair lectin in the initial stage of specific biological recognition arise with regard to R. japonicum/soybean symbioses: About half the strains of R. japonicum do form a capsular polysaccharide that contains D-galactosyl nonreducing end groups, which interact with classical soybean lectin having carbohydrate-binding specificity directed towards N-acetyl-D galactosamine and D-galactose. Many strains, however, are unencapsulated and form an exopolysaccharide composed only of L-rhamnose and 4-O-methyl-D-glucuronic acid. Furthermore, lectinless soybean varieties are nodulated readily.

Soybeans were selected on the basis of preferential nodulation with either of the R. japonicum types. Soybean lectin, extracted from defatted meals of Beeson, Hardee, Lee, and Peking varieties, was isolated by affinity chromatography on immobilized D-galactosamine. Lectin content ranged from 6.2 percent of the total meal protein in Beeson, preferentially nodulated by the galactosyl capsular polysaccharide strain, to 2.3 percent in Peking, preferentially nodulated by strains that form the 4-O-methyl glucuronic acid/rhamnose polysaccharide. It had been reported that the lectin present in soybean roots was membrane-associated and required sonication in detergent for extraction. Protein extracts were isolated from roots of axenically grown plants (Beeson variety). Protein concentrations of the extracts corresponded to those reported. No lectin activity was observed, however, when assayed by precipitin reaction with galactosyl exopolysaccharides from R. japonicum. The reported lectin activity had been detected by a sensitive hemagglutination assay.

The isolated seed lectins all reacted with the galactosyl exopolysaccharides from R. japonicum and Lipomyces starkeyi, but not with the 4-O-methyl glucuronic/rhamnose type. Precipitin reactions were observed between the latter and the unadsorbed portions of the meal extracts. A partial hydrolyzate of the polysaccharide, which contained oligosaccharides, caused dissolution of the precipitin bands; complete hydrolysis to rhamnose and methylated glucuronate destroyed this activity. Addition of β-methyl-D-glucoside to the

unadsorbed fraction of Peking meal extract did not prevent it from reacting with the exopolysaccharide. This result tends to rule out involvement of a β-glucan contaminant in the exopolysaccharide preparation. Presence of a hitherto unrecognized lectin in soybean could resolve current difficulties with the lectin/capsule interaction hypothesis.

b. Specific Objective: Continue investigations on structures of exopolysaccharides from R. japonicum and related rhizobia. Fragment the polysaccharides by various procedures; isolate fragments and study their affinity for soybean lectins.

Progress: Further examination of R. japonicum strains revealed that the methylated glucuronic/rhamnose exopolysaccharide predominates among those associated with rhizobitoxine or chlorosis. These strains tend to be moderate or strong free-living reducers of acetylene. Strains that make the galactosyl capsular polysaccharide tend to be negative or weak with regard to ability to reduce acetylene in culture even if they are associated with leaf chlorosis. Two new R. japonicum exopolysaccharides were found. USDA 18 makes one containing rhamnose, xylose, mannose, and galactose; USDA 70 makes an acidic galactoglucan.

An attempt was made to obtain polysaccharide depolymerases from bacteriophages that infect R. japonicum strains that make the methylated glucuronic/rhamnose polymer. No activity was found at phage titers curtomarily used to produce such enzymes. This failure may reflect the noncapsular nature of the polysaccharide.

c. Specific Objective: Investigate structures of R. japonicum strain 123 and related type exopolysaccharides as well as the L-rhamnose/4-O-methylglucuronic type.

Progress: Structural analysis by deuterioreduction, trideuteriomethylation, and GC/MS of the fragmentation products as per-O-acetyl alditol and aldononitrile confirmed our previous characterization of branched pentasaccharide obtained at the Kettering Laboratory from R. japonicum USDA 138 capsular polysaccharide. The Kettering group has claimed that the product is a tetrasaccharide. A number of new methods were investigated for use in polysaccharide structure analyses. Both polar and nonpolar capillary GC columns successfully resolved a mixture of 14 of the possible 15 O-methylated per-O-acetyl aldononitrile derivatives of D-galactose. Analysis of unmethylated polysaccharides by this type of derivative was improved by use of Silar 5 CP GC columns, substitution of ethyl acetate for chloroform as an extractant, and pyridine removal with aqueous sulfuric acid. Uronic acid components could be estimated, following methanolysis, by GC of their methyl glycosides as trimethyl silyl ethers in either free acid or methyl ester form.

Esterification of alkali metal salts of uronic acids with phenacyl bromide in aprotic solvents and subsequent reduction by sodium borohydride leads to quantitative conversion of carboxyl to carbinol. Experiments on polysaccharides containing uronic acids are giving high

degrees of reduction. It is anticipated that the new procedure will replace in our laboratory the use of ethylene oxide and carbodiimide procedures. Also, it will be economical in the consumption of sodium borodeuteride.

Endo- and exo-β-(1→3)-glucanases from a strain of Cladosporium resinae readily removed β-(1→3)-glucans from preparations of R. japonicum exopolysaccharide.

d. Specific Objective: Develop optimum conditions for growth of R. japonicum and production of exopolysaccharides.

Progress: The medium initially employed for growing strains of R. japonicum produced characteristic polysaccharide relatively free of coproduced β-(1→3)-glucan. An early drop in pH, however, terminated the fermentation after utilization of less than a third of the mannitol carbon source. It was reasoned that addition of a gluconate salt to the medium would counter the decline in pH when gluconate would be metabolized. The decline in pH was, indeed, arrested by replacing 10 percent of the mannitol with potassium gluconate. Surprisingly, it was found that gluconate is preferentially consumed within the first 24-48 hours and little polysaccharide is produced. Subsequently, the mannitol is consumed with production of polysaccharide. Levels of lysine, leucine, isoleucine, and valine, which actually increase during the initial period, now decline.

Monitoring substrate utilization by GC revealed that strains USDA 94 and 123 process mannitol in different ways. (They consume it at equivalent rates and produce different polysaccharides.) Strain 94 first transforms the mannitol into a reducing hexose tentatively identified as fructose by GC/MS. At low phosphate levels, the hexose is not utilized and polysaccharide formation slows appreciably; in high phosphate, formation continues until the hexose is consumed. In contrast, strain 123 secretes no more than trace quantities of the hexose, regardless of phosphate level, and forms two to three times more polysaccharide. Strain 123 is a highly competitive strain indigenous to many Midwest fields where soybeans have been grown previously. The difference in mannitol processing is further evidence for two subspecies of R. japonicum.

Unwanted glucan synthesis has been suppressed by increasing levels of of Mo, Mn, thiamin, and biotin.

Publications:

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EDWARDS, K. G., H. J. BLUMENTHAL, M. KHAN, AND M. E. SLODKI. Intracellular Mannitol, A product of Glucose Metabolism in Staphylococci. J. Bacteriol. 146 (1981):1020-1029.

Other Reports:

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SLODKI, M. E. Disaccharide Phosphate Oligomers from Hansenula capsulata O-Phosphonomannan. Presented at American Chemical Society meeting, Atlanta, Georgia, March 29-April 3, 1981.

2. Physiology of Nitrogen-Fixing Blue-Green Algal and Rhizobial Symbioses with Plants (J. W. Newton)

a. Specific Objective: Study any unique proteins elaborated during development of Azolla blue-green algal nitrogen-fixing symbioses.

Progress: Improvements were made in a high-resolution radio-autographic method for visualizing plant and algal proteins separated by isoelectric focusing-electrophoresis. Algal preparations are resolved into many more components than is plant material, which has been difficult to resolve by this method. Even so, comparisons can be made and characteristic patterns have been obtained for extracts from nitrogen-fixing plants, algal-free plants, algal bundles from plants, and isolated algae grown in culture media. These radioautographic patterns are being compared to seek possibly unique components. The presence of algal spore components in nitrogen-fixing plants confirms the abundance of senescent algae in the leaves.

b. Specific Objective: Isolation of blue-green algae from worldwide collection of Azolla species.

Progress: Although we previously were able to isolate several algal strains from Azolla caroliniana, for unknown reasons, isolation of algae from other species has not yet been achieved. Several associated algae have, however, been isolated from plants and paddy soils. Some of these strains show properties of possible agronomic interest in wetland systems. One strain, which appears to be a Nostoc type, has the unusual property of forming akinetes (spores) and displaying high nitrogenase activity simultaneously. This strain has been found repeatedly in paddy soils. It also forms a high-nitrogen polymer (cyanophycin) of aspartic acid and arginine, which could add large amounts of available nitrogen to such paddys. This polymer is formed subsequent to sporulation and has been detected by electrophoresis of $^{14}C$-labeled material in free-living algae, nitrogen-fixing Azollae and algal bundles from leaves (fronds) of Azolla. Light-dependent evolution of $H_2$ diminishes precipitously during the senescence period. This decrease may reflect more efficient operation of the nitrogenase system.

c. Specific Objective: Study the life cycle of Azolla mixicana to determine the fate of the algal symbiont.

Progress: The massive algal bundle inoculum required for successful isolation of the symbiont of A. caroliniana suggests that akinete germination was involved. Cold treatment (4°C) of the various Azolla species appears to generate the maximum number of akinetes in the symbiotic Anabaena. Crushed leaf preparations of the cold-treated Azolla plants were examined for germination of akinetes. Mild heat shock (34-35°C) of these preparations appeared to trigger initial outgrowth, but development did not progress beyond one division.

d. Specific Objective: Study the pleiomorphic cells of acetylene-reducing rhizobia to determine their relationship to the activity.

Progress: Growth of cowpea-type Rhizobium sp. 32H1 and R. japonicum USDA 26 and 110 on a glutamate-mannitol-gluconate (GMG) agar medium elicited an increase of pleiomorphic cells coincident to acetylene-reducing ($AR^+$) activity. As observed by phase-contrast light, scanning electron, and transmission electron microscopies, the pleiomorphs appeared as if nonuniformly inhibited in cell division. Branched (V, Y, and T shapes) and normal, rod-shaped cells were formed. In contrast, strain USDA 10 was consistently $AR^-$ even though it could produce pleiomorphs during growth under various conditions. When the cell division inhibitors naldixic acid and novobiocin were added (5 µg/ml GMG medium), uniform but $AR^-$ pleiomorphs were formed. This result suggests that distortion of cell division per se is not related to expression of $AR^+$. It should be noted that, in root nodules, the bacteroids are a mixture of pleiomorphic types.

e. Specific Objective: Develop conjugation systems that will transfer acetylene-reducing activity among auxotrophs of Rhizobium japonicum and cowpea-type strains.

Progress: Enrichment cultures of R. japonicum USDA 26 in a N-limited medium containing tryptophan yielded mutant isolates that appeared to metabolize the amino acid. Some of the isolates produce a distinctly tan-colored ($tan^+$) pigment. When grown on GMG, these isolates display a level of acetylene reduction activity similar to that of the parent strain, but much less when the medium is supplemented with tryptophan. In contrast to Klebsiella aerogenes, which utilizes such aromatic amino acids as a sole source of N (Paris and Magasanik, J. Bacteriol. 145:257, 1981), tryptophan plus α-ketoglutarate supported only slight growth of $tan^+$ in a minimal medium. $AR^+$ mutants which yield defined metabolic products could also be useful donor strains of nif genes that would be expressed asymbiotically.

Publications:

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Other Reports:

KANESHIRO, T., F. L. BAKER, AND M. E. SLODKI. Pleiomorphism of Free-Living Rhizobia Grown on Glutamate-Containing Agar Medium. Plant Physiol. (Suppl.) 67 (1981):81.

3. Nitrogen Contribution of Azolla spp. in Aquatic Farming Systems (Cooperative Agreement - University of Hawaii)

a. Specific Objective: Development of Azolla management methods for wetland agricultural systems.

Progress: The nitrogen contributions of various Azolla species; i.e., caroliniana, filiculoides, umbricata, microphylla, and rubra to paddy rice has been studied. The overall mean of N contributions was 142 kg/ha post planting, and 185 kg/ha with combined pre- and post-planting applications. Appropriate combination of Azolla species and management practice was demonstrated to supply enough fixed N in 3 weeks to provide all the N required by a high-yielding rice crop. An Azolla strain has been identified that will survive in water temperatures of 40°C.

b. Specific Objective: Isolation and characterization of blue-green algae associated with Azolla.

Progress: Populations of blue-green algae have been isolated from fields of taro and lotus which contain Azolla. The algae are found in the soil rather than in flood water and belong either to the genera Nostoc or Anabaena. In addition, certain green algae have been isolated that appear to limit the nitrogen-fixing potential of the symbiosis by inducing necrosis of the plants. The significance of this antagonism is under study.

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LUMPKIN, T. A. An Introduction to Azolla niolotica. Acta Botanica Sinica. In press.

Other Reports:

LUMPKIN, T. A. Progress Report on Sino-American Azolla Research. Presented at Academy of Sciences, Peking, People's Republic of China, December 1980.

## B. BIOLOGICAL AGENTS FOR PEST CONTROL

1. Insecticidal Preparations of Bacillus thuringiensis and Other Microbial Insect Pathogens (R. W. Detroy)

a. Specific Objective: Characterize sporulation mutant isolates of B. thuringiensis according to nutritional requirements and potential enzyme deficiencies.

Progress: Protease production was examined among four asporogenic isolates of B. thuringiensis, the wild type, and also two strains of B. thuringiensis var. israelensis. Extracellular protease levels were comparable in all four mutants of B. thuringiensis var. kurstaki and equivalent to the wild type; however, extracellular protease was tenfold less in extracts of B. thuringiensis var. israelensis.

Publications:

JOHNSON, D. E. Toxicity of Bacillus thuringiensis Entomocidal Protein Toward Cultured Insect Tissue. J. Invertebr. Pathol. 38 (1981):94-101.

JOHNSON, D. E. AND B. FREEDMAN. Toxicity of Bacillus thuringiensis Spo$^{-}$ Cr$^{+}$ Mutants for the European Corn Borer Ostrinia nubilalis. Appl. Environ. Microbiol. 42 (1981):385-387.

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Other Reports:

JOHNSON, D. E. Development and Bioassay of Bacillus thuringiensis Sporulation Mutants. Presented at U.S Grain Marketing Research Center, Manhattan, Kansas, August 4, 1981.

## C. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Rapid Characterization of Yeasts Through Genetic and DNA/DNA Hybridization and Computer Analysis (C. P. Kurtzman)

   a. Specific Objective: Determine the extent of DNA relatedness among saturn-spored yeast species.

   Progress: Saturn-spored species from Hansenula and Pichia were compared. H. mrakii and P. sargentensis were found to share 68 percent DNA relatedness, typical for the lower limit of relatedness found between strains of the same species. Consequently, the Saturn-spored species need to be combined into a single genus rather than being separated into separate genera on the basis of nitrate assimilation as was done for the past 50 years. Further H. californica and H. dimennae were shown to be the same species, and H. saturnus var. saturnus and H. beijerinckii were also found to be conspecific.

   b. Specific Objective: Critically examine several yeast strains that appear new to science.

   Progress: One isolate, previously classified as Pichia saitoi, was shown by DNA relatedness studies to differ from P. saitoi and, therefore, represent a new species.

Publications:

KREGER-VAN RIJ, N. J. W. AND C. P. KURTZMAN. Principles of Classification--Classification of Ascosporogenous Yeasts. In The Yeasts, A Taxonomic Study, 3rd Ed., ed., N. J. W. Kreger-van Rij, North-Holland Publishing Co., Amsterdam, The Netherlands. Accepted.

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Other Reports:

KURTZMAN, C. P. Restoration of the Genus Williopsis for Classification of Saturn-Spored Yeasts Presently Assigned to Hansenula and Pichia. Presented at American Society for Microbiology meeting, Dallas, Texas, March 1-6, 1981.

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KURTZMAN, C. P. Changes in the Taxonomy of Ascomycetous Yeasts. Presented at U.S. Federation for Culture Collections, American Institute of Biological Sciences, meeting, Bloomington, Indiana, August 16-20, 1981.

2. Germ Plasm Bank of Microorganisms for Research on Plant Residue Utilization (C. P. Kurtzman)

a. Specific Objective: Continue operation of the Agricultural Research Service Culture Collection (NRRL) including original and supportive research.

Progress: Microbiology staff members of the Agricultural Research Service Culture Collection (NRRL) continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their original and supportive research. As of January 1, 1982,

the Collection maintained 72,445 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1981, the Collection distributed 4,251 strains, of which 2,832 were provided to investigators in the United States and 1,419 were sent abroad. Of 183 strains deposited in the patent collection, 88 were from foreign sources; 368 patent strains were distributed to United States researchers, and 299 to foreign. One-hundred and seventy strains of bacteria and actinomycetes were identified to species for interested parties.

One strain of a nonlegume nitrogen-fixing actinomycete was studied to determine its growth characteristics and taxonomy. A strain of Frankia sp. was found to grow very slowly on most nutrient media and the biomass produced was insufficient to determine most characteristics. However, it was found that the cell walls contained the meso isomer of diaminopimelic acid. Lowering the oxygen content of the atmosphere gave a very slight increase in the biomass production. It was found that the strain could be lyophilized and preserved.

Early in 1981, the Agricultural Research Service Culture Collection (NRRL) and the American Type Culture Collection (ATCC) were designated as International Depositary Authorities for the deposit of patent strains in connection with the World Intellectual Property Office (WIPO) and the Budapest Treaty. The necessary international deposit, receipt, and viability forms have been prepared and the deposit forms have been distributed to the depositors. In addition, the French Patent Office, in August 1981, recognized us as an international depository.

Chemical analyses of wheat straw and wood shavings fermented by different species of Cyathus (birds nest fungi) were nearly completed.

b. Specific Objective: G+C determination and DNA reassociation studies of agriculturally significant bacteria will be continued.

Progress: Studies of 112 Bacillus circulans strains continued to show that this group partitioned into three groups on the basis of G+C contents of the DNA. Of the strains examined, 19 percent had G+C contents of 38-40 mol percent (the type strain fit into this group), 56 percent G+C contents of 48-50 mol percent, and 25 percent G+C contents of 52-54 mol percent. Within the group having G+C contents of 38-40 mol percent, 10 of 16 strains showed 90-100 percent DNA relatedness to the type strain and among themselves. Incomplete studies of the higher G+C groups indicated high DNA relatedness only among a relatively small number of strains.

Preliminary studies indicate that the organisms having G+C contents of 38-40 mol percent and showing high DNA relatedness are phenotypically homogeneous.

c. Specific Objective: Preparation of a comprehensive compendium of methods for the preservation of microorganisms, cell lines, and viruses will be continued.

Progress: First drafts of several chapters have been prepared.

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PRIDHAM, T. G. Recombinant DNA Technology: Guidelines and Effects on Culture Collections and Patents--Introductory Remarks. Presented at American Society for Microbiology meeting, Dallas, Texas, March 1-6, 1981.

3. Effect of Immobilization Procedure and Carrier on Enzymes that Hydrolyze Cereal Food Polymers (M. E. Slodki)

a. Specific Objective: Prepare hydrolytic fractions of various glucans.

Progress: Samples of yeast "glucan" and a polysaccharide preparation obtained from a strain of Bacillus subtilis were provided by the Beltsville Agricultural Research Center (BARC). These polysaccharides had been shown by BARC to inhibit sugarcane mosaic virus symptoms on sorghum. We quickly found that the yeast polysaccharide is actually a mannan, not a glucan. Methylation/fragmentation analysis gave the products expected of Saccharomyces cerevisiae mannan. The Bacillus polysaccharide is also mannan-like. The component sugars are D-mannose:D-glucose:D-galactose in respective molar ratios of 6:1:1. Methylation analysis awaits receipt of a more purified preparation from BARC.

In view of the foregoing, a series of 14 extracellular mannans, glucomannans, and O-phosphonomannans were sent to BARC. The preparations are being screened against sugarcane mosaic and bean common mosaic viruses.

b. Specific Objective: Metabolic products and pathway utilized by Pachysolen tannophilus when converting xylose to ethanol.

Progress: Because P. tannophilus is the only yeast known to "ferment" a pentose to alcohol, it has assumed considerable interest for conversion of biomass to ethanol. Studies conducted at NRRC by personnel in Biomaterials Conversion Laboratory and the ARS Culture Collection Research group established that growth of the organism on xylose could only be initiated if air was present, that alcohol yields were less than 85 percent of theory and that an organic acid was one of the fermentation products.

Attempts were made to identify the acid by ether extraction of acidified, fermented beers. The ether extracts chromatographed on a μBondapak $C_{18}$ column, preequilibrated with $0.01M H_3PO_4$, consistently showed a compound eluting at about 5.3-5.4 minutes. The column efficiently separated known di- and tricarboxylic acids as well as functional acids such as lactic, glyceric, and phosphoglyceric. Oxalic acid elutes at the same time as the unknown but was eliminated when calcium chloride failed to form a precipitate. It also elutes close to, but not exactly the same, as phosphoglyceric acid. Phosphoglyceric or one of the closely related phospho acids of the Meyerhoff-Embden scheme are a likely possibility since it is presumed that alcohol formation takes place by this pathway.

Volatile monocarboxylic acids are not present because no acid was found in condensates from steam distillation of acidified P. tannophilus beers. The quantity of acid extractable by ether is not enough to account for all the products other than alcohol that are formed from xylose.

The bulk of the carbon unaccounted for is apparently in the form of a mannan that can be precipitated by alcohol. It has long been established that P. tannophilus forms either a phosphomannan or a mannan under aerobic conditions from glucose. If the organism is utilizing the pentose phosphate shunt mechanism then the formation of such polysaccharides would be expected.

Some progress has been made on the metabolic pathway utilized by P. tannophilus to convert xylose to a fermentable substrate. A number of yeasts (such as strains of Candida) are known to aerobically consume xylose as an energy source for cell production. For this purpose, they reduce xylose to xylitol via NADPH. Xylitol is, in turn, oxidized by xylulose by NAD. Xylulose is then phosphorylated by a phosphokinase and the metabolism proceeds from there by the pentose phosphate shunt mechanism. As stated earlier, P. tannophilus does require some air to initiate growth. Therefore, a study was initiated to see if this organism has both an NADP-dependent xylose reductase and an NAD-dependent xylitol dehydrogenase.

Cells of P. tannophilus were grown in Fernbach flasks in a xylose medium under aerobic conditions. The cells were harvested and ruptured in a French press. The cell-free preparations were used as an enzyme source. A very active NAD-dependent xylitol dehydrogenase was demonstrated by following the conversion of NAD and NADH spectrophotometrically. The enzyme was present in all six cultures of P. tannophilus available to us.

To demonstrate the NADP-dependent xylose reductase, the reaction was run with xylose and NADPH as the coenzyme. Again, all six strains showed xylitol formation from xylose although the rates were not as fast as the xylitol to xylulose reaction. No xylitol dehydrogenase could be demonstrated in glucose-grown cells of P. tannophilus.

The question now remains as to why P. tannophilus forms alcohol from xylulose under mildly aerobic conditions rather than cell substrate as in the case of Candida yeasts. At present it can only be postulated that P. tannophilus is a "respiration deficient" yeast and, therefore, must revert to fermentation to get the necessary ATP for growth and maintenance.

Another possible route from xylose to xylulose is by isomerization of xylose with xylose isomerase. We were unable to demonstrate such a reaction in P. tannophilus as no ketopentose sugars were detectable by the cysteine-carbazole reaction.

c. Specific Objective:

Products of enzymic degradation of xanthan gum.

Progress: Work on the biodegradation of xanthan gum (polysaccharide B-1459) was completed when the low-molecular-weight products were identified. These products, as separated by paper chromatography and characterized after elution, were D-mannose, D-glucuronic acid, pyruvylated mannose, and 6-O-acetyl-D-mannose. Location of the O-acetyl substituent was accomplished by substituting acetic anhydride-$d_6$ in the procedure for making per-O-acetyl aldononitrile derivatives and examining the mass fragmentations. This research demonstrated that the derivatization procedure does not disturb preexisting O-acetyl substituents. The xanthanase is clearly a complex of enzymes that individually remove the side-chain sugar units of xanthan gum.

Publication:

SMILEY, K. L., J. A. BOUNDY, AND D. E. HENSLEY. Action Patterns of Immobilized Dextranase. Carbohydr. Res., accepted for publication.

4. Characterization and Classification of Mucorales from Cereal Grains and Their Raw Products (C. W. Hesseltine)

a. Specific Objective: Collect and preserve isolates of Rhizopus and related genera of the Mucorales from various materials, especially cereal grains and their products.

Progress: A total of 76 new strains of Rhizopus and 15 related genera were collected from a wide variety of sources such as brewery material, carpet, feed supplement, herbivore dung, hog feed, Nepal yeast, patients, and sunflower heads. Three isolates were our first representatives of the recently described genus Apophysomyces previously known only from India. Because of parallels of its growth pattern with that of Saksenaea, a method that was developed for stimulating sporulation in the former also increased sporulation of nearly all strains tested of the latter.

b. Specific Objective: Classify strains of Rhizopus sp.

Progress: In light of the previously found variation in spore size of Rhizopus delemar due to media and temperature, 170 strains identified as R. oryzae in the collection have been revived from lyophil storage and examined on appropriate media at 25° ad 37°C. Most of the strains appeared to retain the current species concept of R. oryzae, nearly 10 percent were reidentified as R. arrhizus, whereas 20 percent of the strains remained intermediate between the two species and require a different approach to determine speciation.

When 50 strains representing the type and authentic strains for species of Rhizopus were grown on raffinose of inulin as sole carbon sources, there was not a clear grow-no grow situation between species or groups of species as indicated by Hanzawa in 1915. This physiological characteristic distinguishes species such as R. trubini from R. usamii or R. oryzae from R. arrhizus as he did. Dry weights of the growth for the 50 strains could be aligned in a near continuum for each carbon source.

c. Specific Objective: Investigate an unusual strain of Cokeromyces.

Progress: Morphological data and characteristics of the unusual strain of Cokeromyces were gathered, consolidated, and evaluated in comparison to Cokeromyces recurvatus, C. poitrasii, Mycotypha africana, and M. microspora. Because the data did not convincingly support either keeping C. poitrasii in the genus or removing it to the genus Mycotypha, further efforts were made to restimulate lost sporulation in the unusual strain as well as attempt to increase the rate and amount of growth. Various combinations of ingredients of the three best growth media and addition of buffering at pH 7.0 or addition of thiamine did not increase growth rate nor restimulate sporulation.

d. Specific Objective: Investigate the Mucoraceous molds in ragi.

Progress: During the past year, 17 samples of ragi and Chinese yeast have been examined for their total counts for Mucorales and yeasts. Selected cultures from the samples have been incorporated into the Culture Collection. It has been found that all the Mucorales in the ragi or ragi-like products belong to the genera Rhizopus, Amylomyces, Absidia, and Mucor.

Publications:

ELLIS, J. J. The Effect of Medium, Temperature, and Age on Rhizopus delemar Sporangiospore Size. Mycologia 73 (1981):262-368.

ELLIS, J. J. AND L. AJELLO. An Unusual Source for Apophomyces and a Method for Stimulating Sporulation of Saksenaea. Mycologia. Accepted.

5. Molecular Genetics Technology for Microbial Production of Plant Polysaccharide-Degrading Enzymes (R. W. Detroy)

a. Specific Objective: Develop methodology for effective screening, isolation, and characterization of extrachromosomal DNA in fungi.

Progress: One strain each of Aspergillus nidulans and Penicillium funiculosum were broken gently with a Waring blender. The nuclei and mitochondria were removed from the homogenates via centrifugation and the extra-nuclear supernates extracted for total nucleic acids. The RNA was removed with RNase and the residual DNA analyzed by agarose gel electrophoresis. No plasmids were detected. No detectable plasmids were found in the xylose-fermenting Pachysolen tannophilus using CsCl-ethidium bromide centrifugation followed by gel electrophoresis.

b. Increase microbial starch-degrading/xylose-utilizing enzyme production through employment of cell fusion technology and selective mutational techniques.

Progress: Fusion of Pachysolen tannophilus with Saccharomyces cerevisiae has been accomplished by traditional and chemical induction methods. Fusion of chemically treated protoplasts has produced, for the most part, genetically unstable cells, although the more stable cells are presently being tested. A relatively stable (~85%) cell line produced by traditional genetic recombination between the two genera is being analyzed to determine if this clone is a spontaneous mutant or is indeed a fusion product.

The techniques for transforming Escherichia coli are being worked out and a gene bank of P. tannophilus DNA is being prepared. The present rate of transformation (~0.1%) is too low to effectively establish a bank which would include the entire nuclear genome of P. tannophilus.

c. Specific Objective: Transformation of the cellulase enzyme complex genes into yeast/bacteria for the production of fermentable sugars from cellulose.

Progress: Ten selected isolates of Penicillium funiculosum and six selected isolates of Aspergillus nidulans were grown on a salts media with cotton as the sole carbon/energy source. After 4 weeks' growth at 28°C, the culture beers were harvested and analyzed for extracellular cellulase production. None of the A. nidulans strains produced the complete cellulose complex ($C_x$-, $C_1$-enzymes, cellobiose). Seven of the P. funiculosum strains produced the complete complex, with NRRL 3503 producing the most activity. Nuclear DNA from NRRL 3503 and E. coli plasmid pBr 322 have been isolated. Experiments are currently in progress to make a P. funiculosum pBr 322 library and screen this library for the P. funiculosum $C_x$ and cellobiase genes.

As an alternate source of the cellobiase gene, 14 yeasts which have been previously reported to be able to ferment cellobiose were tested for EtOH production capacity on 9-percent cellobiose. The two most active organisms (Torulopsis wickerhamii and Candida lusitaniae) were able to produce 2.5 percent and 4.4 percent EtOH in 7 days. From analysis of the fermentation beers, T. wickerhamii also converts essentially all of the unfermented cellobiose to glucose; thus, the final beer contains 2.5-percent EtOH and 5-percent glucose after 7 days. This conversion of cellobiose to glucose appears to be due to an overproduction of cellobiase by T. wickerhamii. Partial cloning of the Aspergillus nidulans genome has been established in the lambda (λ) phage virus. Twelve kilobase fragments have been isolated by restriction of the isolated fungal DNA. The fragments were cloned into a λ phage packaging system for establishment of a fungal genome bank. The viral clone bank can now be tested for the transformation of cellulase-degrading genes into a bacterial/yeast system for expression.

Publications:

DETROY, R. W. Fermentation of Plant Polysaccharides: Role of Biochemical Genetics. In: "Trends in the Biology of Fermentations for Fuels and Chemicals," Edited by A. Hollaender, pp. 183-185, Plenum Press, New York, 1981.

FREER, S. N. Fungal Nucleic Acids. In: "Secondary Metabolism and Differentiation in Fungi," Edited by J. W. Bennett and A. Ciegler, Marcel Dekker, Inc., New York. In press.

ALEXANDER, N. J. AND R. W. DETROY. Application of Biochemical Genetics: Fermentation of Cellulose/Hemicellulose to Ethanol. International Symposium Genetic Engineering, Sao Paulo, Brazil. In press.

Other Reports:

DETROY, R. W. Application of Recombinant DNA Research: Plant Polysaccharide-Degrading Enzymes and Alcohol Fuels. Presented at Conf. General Cooperators, NCR, Peoria, Illinois, October 19-20, 1981.

ALEXANDER, N. J. Alcohol Production by Wild-Type and Mitochondrial Mutants of Saccharomyces cerevisiae. Presented at Genetics Society of America meeting, Raleigh, North Carolina, June 15-17, 1981.

DETROY, R. W. Fermentation of Plant Polysaccharides. Role of Biochemical Genetics. Presented at Trends in the Biology of Fermentations Symposium, Brookhaven National Laboratories, Upton, New York, December 6-10, 1980.

DETROY, R. W. Molecular Cloning Applications to Fermentative Microorganisms. Presented at Lignocellulosic Chemistry Workshop, San Felipe, Venezuela, September 4, 1981.

DETROY, R. W. Application of Recombinant DNA Technology to Microbial Fermentations. Presented at Vegetable Oil as Diesel Fuel, Seminar II, NRRC, Peoria, Illinois, October 21, 1981.

ALEXANDER, N. A. Genetic Engineering of Yeast for the Production of Ethanol. Presented at University of Iowa, Iowa City, Iowa, November 2, 1981.

ALEXANDER, N. A. Genetically Engineered Microbes for Bioconversion Processes. Presented at Western Illinois University, Macomb, Illinois, October 2, 1981.

ALEXANDER, N. A. Applications of Recombinant DNA Research. Presented at Illinois Society for Microbiology, Normal, Illinois, May 19, 1981.

ALEXANDER, N. A. Split Genes in the Mitochondrial DNA of Saccharomyces cerevisiae. Presented at Western Michigan University, Kalamazoo, Michigan, February 18, 1981.

6. Conversion of Cellulosic Wastes into Feed for Ruminants (P. L. 480 Grant - Pakistan Council of Scientific and Industrial Research Laboratories, Lahore)

a. Specific Objective: Feeding experiments using treated wheat straw, bagasse pith, and rice straw.

Progress: Microorganisms capable of growing on crop residues were isolated from Pakistani soil and identified. Four strains of mold (Chaetomium globosum, Trichoderma viride, Penicillium roqueforti, and Streptomyces griseus) and seven strains of bacteria (Bacillus subtilis, B. brevis, B. laterosporus, B. polymyxa, B. pumilus, B. sphaericus, B. cereus), and yeast (Saccharomyces cerevisiae) grew well when crop residues were used as source of carbon. Single and symbiotic effect of bacteria, molds, and yeast were studied using bagasse, wheat and rice straws, and beet pulp feed as substrate by submerged fermentation process. Chaetomium biodegraded 13.04 percent of the cellulose present in bagasse and 16.01 percent wheat straw whereas B. cereus biodegraded cellulose present in these two residues up to 3.56 percent and 12.62 percent, respectively. An improvement in the digestibility of bagasse and wheat straw was observed due to symbiotic effect of B. cereus and Chaetomium.

In vivo digestibility of sodium hydroxide, ammonium hydroxide, and calcium hydroxide treated and biodegraded bagasse pith, wheat straw,

rice straw, beet pulp feed, and cottonseed hulls was also studied. Treatment with sodium hydroxide showed better results than the other chemicals.

The dry matter digestibility of wheat straw was 50.80 percent due to symbiotic effect of B. polymyxa and Chaetomium in semisolid fermentation process. Maximum dry matter digestibility of biodegraded and alkali-treated bagasse pith, rice and wheat straws was also improved after biological treatment.

Rice hulls were also tried for propagation of oyster mushrooms. Pleurotus was propagated satisfactorily on the hulls and biodegraded 30.3 percent of the cellulose present in the hulls. This was accompanied by an increase in the crude protein percentage from 15.6 to 23.1 and improvement in the digestibility of the hulls from 18.58-33.34 percent after 30 days of mycelial growth.

Bagasse pith and fresh cow dung were ensiled, in laboratory type silos, for a period of 33 days. The silage was free from pathogenic bacteria, contained up to 2.94 percent lactic acid and showed up to 9.4 percent increase in protein and up to 8.3 percent in ether extracts. The increase in dry matter digestibility was up to 22 percent.

The final report was received from grantee in December 1981.

7. Fermentative Utilization of Cane Sugar Bagasses (P. L. 480 Grant - National Research Centre, Cairo)

   a. Specific Objective: Utilization of cane sugar bagasses.

      Progress: Saccharification studies indicated the suitability of Trichoderma viride 253 crude enzyme preparation as a promising agent for saccharifying bagasse hemicellulose, treated α-cellulose, and alkali-treated bagasse. Utilization of sugarcane bagasse for the fermentative production of cellulases, hemicellulases, and SCP by T. viride 253 can be outlined as follows: (1) Production of extracellular cellulases and hemicellulases in a forced aeration-stirred tank fermentor using crude bagasse (CB) as the sole carbon source in Dox's culture medium. (2) Treatment of the remaining biodegraded bagasse (BB) with NaOH. (3) Refermentation in static culture of bagasse as the sole carbon source in Dox's culture medium for production of SCP material.

## D. TECHNOLOGIES FOR FOOD AND FEED USES FOR ANIMAL PRODUCTS

1. Conversion of Feedlot Wastes into Feed Supplements by Fermentation with Grain (G. R. Hrubant)

   a. Specific Objective: Complete pathogen-free capabilities of the continuous fermentation of corn with cattle waste.

      Progress: Five bacterial virus populations were substantially reduced in numbers during the fermentation. Fewer than eight viruses per

10,000 added in the first chamber were recovered in the output over a 5-day interval, 78% of these during the first 36 hours of operation. Were the output allowed to accumulate over the 5-day period, the total viable numbers in the product would be reduced another hundredfold. With initial inocula of $10^8$ to $10^{10}$ viruses/g total material in the first chamber, the numbers of viable viruses decreased 90% per time interval given: 18.1 hours for ZIK/1 (1-RNA, 22.5 nm smooth icosahedron), 15.6 hours for ZJ/2 (1-DNA, 5.5 X 850 nm filament), 8.3 hours for ØX174 (30 nm icosahedron with 12 large apical capsomeres), 11.7 hours for PL-1 (2-DNA, 63 nm icosahedral head with 275 nm noncontractile tail), and less than 5 minutes for total kill of Ø6 (2-RNA, 60 nm icosahedron with lipid envelope). Controls (1/4 strength autoclaved feedlot waste liquid) were essentially stable for the 5-day period.

b. Specific Objective: Initiate comparison of microbial profiles of cattle wastes from cattle on diets containing/not containing rumensin.

Progress: Antibiotic resistance profiles were established for microbes in waste from young, pastured cattle after a 2-week period of silage feeding in pens. Rumensin and other antibiotics were not given to the animals. Total aerobes, yeasts, fecal coliforms, lactic acid bacteria, and "Arthrobacters" (defined only by growth on Owens and Keddie medium) were enumerated. Numbers of organisms resistant to low levels of rumensin and 10 other antibiotics were determined. Total aerobes numbered 5.6 X $10^9$/g wet weight manure. One-eighth were resistant to rumensin (2 mcg/ml); three-fourths were resistant to streptomycin (2 mcg/ml), and one-fourth to neomycin (5 mcg/ml). Approximately 5% were resistant to ampicillin (2 mcg/ml) or to penicillin G (2 units/ml). All yeasts, 7.0 X $10^5$/g, and fecal coliforms, 1 X $10^7$/g, were resistant to 100 mcg/ml of rumensin. Additionally, over half of the fecal coliforms were resistant to streptomycin, tetracycline, aureomycin, and ampicillin. Fewer than 300 fecal coliforms/g were resistant to chloramphenicol (5 mcg/ml) or polymyxin B (50 units/ml). The waste contained 8.8 X $10^6$ lactic acid bacteria per gram. Only 2% were resistant to rumensin, a large proportion being Leuconostoc rather than lactobacilli. Essentially all lactics were resistant to low levels of streptomycin and neomycin. Three-fourths of the lactics were resistant to polymyxin B and 30-40% were not inhibited by tetracycline, aureomycin, or vancomycin (5 mcg/ml). Ten percent were resistant to penicillin G but only 1% survived ampicillin. Only 5% and 0.04% of the lactics were resistant to bacitracin (2 units/ml) and chloramphenicol, respectively. "Arthrobacters" numbered 4.4 X $10^9$ organisms per gram. Forty-one percent were resistant to rumensin and one-half were unaffected by streptomycin. Fourteen to 22% were resistant to neomycin, penicillin G, ampicillin, or bacitracin. Resistances to tetracycline, aureomycin, chloramphenicol, vancomycin, or polymyxin B varied from 0.3 to 5%. Resistance spectra to the 11 antibiotics are being determined for bacterial isolates from all antibiotic-free and rumensin-containing media.

c. Specific Objective: Investigate fecal coliform removal in an anaerobic process of swine waste combined with corn.

Progress: Swine waste combined with corn in a laboratory silo initially contained $5.41 \times 10^5$ fecal coliform bacteria. These organisms decreased in number to $2.04 \times 10^2$ in 24 hours and were never again detected through a fermentation period of 6 months.

d. Specific Objective: Investigate changes in population of lactic acid bacteria in an anaerobic process which combines swine waste with corn.

Progress: The total number of microorganisms at the start of fermentation ($10^7$/dry g) was dominated by 80% lactic acid bacteria. These were lactobacilli which divide into three groups: The homofermentative streptobacteria and thermobacteria present in quantities of 77 and 3%, respectively, and the $CO_2$-producing heterofermentative betabacteria present in 13% abundance. Streptobacteria remained as the dominant group of lactobacilli through 6 days, but betabacteria decreased to 2%. These facultatively anaerobic lactobacilli grew through 38 days and then died, leaving an anaerobic lactobacillus to ferment at number of $10^3$/dry g through 90 days.

e. Specific Objective: Contrast numbers of viable microorganisms (total, lactic acid bacteria, and coliforms) with respect to aerobic and anaerobic conditions of growth.

Progress: Total microbial numbers (per dry g) of the start of anaerobic culture was $10^7$ and a tenfold increase occurred at 24 hours. A virtually similar pattern of growth of facultative anaerobes and anaerobes with decreasing numbers occurred until 9 days. Comparable numbers of anaerobes and facultative anaerobes show that the latter group is the major part of total organisms. Facultative anaerobes then drop to $10^4$ at 11 days but anaerobes do not drop to this level until 17 days. Thereafter, numbers of both groups of organisms remain at $10^4$ days. Numbers of lactic acid bacteria, primarily lactobacilli, measured with roll tubes, equaled or exceeded that found with plate counts by as much as 100-fold. Facultative anaerobic lactobacilli (plate counts) drop tenfold at 5 days from initial levels of $10^7$ and continue to drop in number to be zero at 41 days. Anaerobic lactobacilli decrease more slowly in number to reach $10^3$ organisms at 41 days and maintain this level through 90 days. Thus, anaerobic lactobacilli and another group of facultative anaerobic g + bacilli represent a modest but enduring microbial population at 90 days.

Report:

HRUBANT, G. R. Kill of Bacterial Viruses, Mycobacterium tuberculosis, and Escherichia coli in the Continuous Fermentation of Corn with Feedlot Waste Liquid. Presented at S-139 Technical Committee meeting, Auburn, Alabama, January 8-9, 1981.

2. Feeding Trials of Animal Waste-Grain Fermented Feeds Using Recycled Waste (Cooperative Agreement - University of Illinois)

a. Specific Objective: Construct and operate a pilot-scale model of continuous fermentor.

Progress: Construction is approximately two-thirds complete. Where possible, parts are being tested before final assembly. With essentially all parts purchased, hardware cost of the fermentor is approximately $2,500. However, all motors are being supplied free by the contractor for the term of the contract. Estimated date for completion of construction is January 1982.

## E. BIOMATERIALS SCIENCE

1. Increased Energy Efficiency of Substrate Preparation for Alcohol Fermentations (R. W. Detroy)

See Northern Agricultural Energy Center, A.2.

2. Innovative Fermentation Technology for Alcohol Production (R. W. Detroy)

See Northern Agricultural Energy Center, A.3.

## F. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

1. Soybean Foods of the Traditional Oriental Type for the Export Market (H. L. Wang)

   a. Specific Objective: Investigate the factors affecting the quality of tofu production.

   Progress: Calcium sulfate, magnesium sulfate, calcium chloride, and magnesium chloride were used as coagulants in this study. Earlier experiments indicated that lowest curd weight, volume, and solid recovery were noted at salt concentrations between 0.02-0.04 M. Further studies showed that salt concentrations at this range had the least effect on these quantities and also resulted in the highest nitrogen recovery. Therefore, the use of salt at a level between 0.02 to 0.04 M is more likely to yield reproducible products with high protein content. For the same reason, calcium sulfate ($CaSO_4 \cdot 2H_2O$) seems to be the preferred coagulant. Anhydrous forms of calcium sulfate and magnesium chloride failed to yield acceptable curd.

   The type and the concentration of salts used for coagulation also affect the texture characteristics of tofu. When the concentration of the salt was increased from 0.01 to 0.02 M, significant increase in hardness, brittleness, cohesiveness, and elasticity of the curds were noted. No significant changes were observed at concentrations between 0.02 to 0.04 M, and above that range these measurements decreased slightly except when calcium sulfate was used.

   Calcium chloride and magnesium chloride resulted in curds with much greater hardness and brittleness than did calcium sulfate and magnesium sulfate, suggesting that anions have a greater effect than cations on these two parameters. There was no significant difference in cohesiveness of the curds made from the four salted investigated indicating cohesiveness may be an inherent texture characteristic of a given protein.

Milk temperature at the time of adding salt also affects the texture, increasing hardness and elasticity as the temperature increases.

b. Specific Objective: Investigate the keeping quality of tofu.

Progress: There was no bacterial count in freshly made tofu and in tofu that has been stored at 4°C for 4 days. After keeping at 4°C for 7 days, a count of $10^4$ per gram of sample was found, and the count increased steadily to $10^8$ after 21 days. Thereafter, no significant change was noted. When tofu was stored at 15°C, the bacterial count reached $10^7$ after 4 days. This storage study emphasizes the importance of refrigeration in obtaining an acceptable shelf life. The most frequently isolated bacteria are strains of Arthrobacter and Bacillus subtilis.

c. Specific Objective: Investigate the effect of storage on the quality of soybeans.

Progress: 1980 Corsoy soybeans at three moisture levels, 8.5, 10.2, and 11.4%, are stored in metal, wooden, or plastic containers and kept at ambient temperatures as practiced by the industry. Samples were taken periodically and tested for moisture content, percentage of germination, protein solubility, trypsin inhibitor, and cooking quality. Moisture content of the beans fluctuated significantly, perhaps with the atmospheric conditions. After 8 months of storage at ambient temperature, the initial differences in moisture content between the samples were reduced, especially for those beans stored in wooden containers, 10.0, 10.6, and 10.7% as compared to the initial content of 8.5, 10.2, and 11.4%, respectively. Percentage of germination and the weight gain of beans after cooking decreased gradually as the storage time progressed. There were no significant changes in protein solubility, trypsin inhibitor, and tenderness of cooked beans. The study will continue to 18 months of storage time.

d. Specific Objective: Initiate a study on amylase produced by microorganisms used in Oriental food fermentations.

Progress: Preliminary investigation indicated that strains of Amylomyces rouxii, Rhizopus chinensus, R. oligospores, and R. arrhizus produce various amounts of amyloglucosidases. The enzymes also attacked the raw starch, although the reaction rate was low. The growth of these molds on rice resulted in products with various aromas. The aroma together with the enzyme production could be used as criteria for selecting the best suitable cultures for rice fermentations.

Publications:

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WANG, H. L. AND C. W. HESSELTINE. Use of Microbial Cultures: Legume and Cereal Products. Food Technol. 35 (1981):79-83.

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WANG, H. L. Nutritional Value of Fermented Foods. Presented at Soycrafters Conference, Colorado State University, Fort Collins, Colorado. July 8-12, 1981.

WANG, H. L. Coagulation Conditions in Tofu Processing. Presented at Soycrafters Conference, Colorado State University, Fort Collins, Colorado. July 8-12, 1981.

HESSELTINE, C. W. Shelf-life of Tofu. Presented at Soycrafters Conference, Colorado State University, Fort Collins, Colorado. July 8-12, 1981.

## G. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Germ Plasm Bank of Microorganisms for Research on Microbial Toxins (D. T. Wicklow)

   a. Specific Objective: Continue operation of the Agricultural Research Service Culture Collection (NRRL) including original and supportive research.

   Progress: Mycology staff members of the Agricultural Research Service Culture Collection (NRRL) continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their original and supportive research. As of January 1, 1982, the Collection maintained 72,445 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1981, the Collection distributed 4,251 strains, of which 2,832 were provided to investigators in the

United States and 1,419 were sent abroad. Of 183 strains deposited in the patent collection, 88 were from foreign sources; 368 patent strains were distributed to United States researchers and 299 to foreign. One hundred and forty-three strains of molds and yeasts were identified to species for interested parties.

Early in 1981, the Agricultural Research Service Culture Collection (NRRL) and the American Type Culture Collection (ATCC) were designated as International Depositary Authorities for the deposit of patent strains in connection with the World Intellectual Property Office (WIPO) and the Budapest Treaty. The necessary international deposit, receipt, and viability forms have been prepared and the deposit forms have been distributed to the depositors. In addition, the French Patent Office, in August 1981, recognized us as an international depository.

b. Specific Objective: Develop new media and evaluate them for stimulation of sporulation by strains of molds in order to preserve abundant germ plasm by lyophilization.

Progress: Fifty nonsporulating or poorly sporulating strains representing 31 genera of the NRRL mold collection were grown on six newly devised media. On one medium, a strain of Colletotrichum formed a compound that crystallized out into the medium, a hard to maintain strain of Emericellopsis was successfully relyophilized from another medium, and a patent strain sporulated and was successfully lyophilized from another medium. Further, a sterilized wheat kernel-grass extract medium was devised that stimulated increased macroconidial production by strains of Fusarium graminearum for inoculum used in vomitoxin studies.

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2. Aflatoxin and Other Mycotoxins in Corn and Other Cereal Grains (O. L. Shotwell)

a. Specific Objective: Study regions and situations in which there are mycotoxins in grains and investigate methods of sample handling after collection.

Progress: A 5-year (1976-1980) study has been completed in which about 100 samples of wheat and of corn were collected by the USDA Federal Grain Inspection Service in Virginia for aflatoxin, zearalenone, and ochratoxin A analysis. Zearalenone, aflatoxin, and ochratoxin A were not detected in any wheat sample. None of the corn samples had detectable zearalenone or ochratoxin A. However, aflatoxin was detected in corn samples harvested every year, but average levels (21-137 ng/g) and incidences (36-86%) varied greatly. Freshly harvested corn samples collected by the Statistical Reporting Service in 1978 and 1979 contained lower average levels (13, 36 ng/g) and incidences (12, 21%). Six sets of matched samples were harvested from each of 57 Georgia corn fields to study effects of storage times and 5% Monoprop (1 propionic acid plus 1 vermiculite) on aflatoxin levels. Aflatoxin average level (217 ng/g) did not increase in 1980 during the average 7 days' transit time between Georgia and Peoria, but doubled in undried samples during 3 weeks' storage after arrival. Monoprop treatment (2.5% propionic acid) decreased during 3 weeks' storage by half aflatoxin levels, possibly by the formation of the hemiacetal.

b. Specific Objective: Continue study on inhalation exposure of agricultural workers to contaminated dust from aflatoxin-containing corn with Dr. William R. Burg, Department of Environmental Health, University of Cincinnati.

Progress: Airborne dust samples were collected on a farm during harvest and at an elevator in Georgia in 1980. The bulk corn harvested on the farm contained 1600 ppb total aflatoxin; airborne dust collected in personal samplers contained 1000-23,000 ng/g with a mean of 18,500 ng/g. Total dust samples average 3000 ng/g. The high levels observed in airborne dust may be accounted for by the spores of the Aspergillus flavus group. The average aflatoxin level in nine species of A. flavus and A. parasiticus was 103,000 ppb. Personal samplers were used to collect airborne dust in elevators; from these results, the quantity of aflatoxin in airborne dust in a given volume of air can be calculated. At the elevator, the average level in bulk corn was 153 ng/g; in total airborne dust samples, 1200 ng/g; and from personal samplers, 668 ng/g.

c. Specific Objective: Develop and evaluate new approaches to aflatoxin analysis of corn and mixed feeds.

Progress: The modified animal tissue method for aflatoxin has been utilized to test four different feeds at three aflatoxin $B_1$ levels in triplicate. Average recoveries are as follows: turkey starter, 92%; catfish chow, 97%; pig starter, 95%, and broiler finisher, 103%. Two additional feeds are being tested.

d. Specific Objective: Develop and evaluate rapid and quantitative methods for analyzing cereal grains and feeds for mycotoxins, perticularly Fusaria toxins.

Progress: A method was developed for the extraction and quantitation of deoxynivalenol from field-inoculated corn and *in vitro* fermented substrates. This procedure involves the extraction of deoxynivalenol with methanol:water (50:50 v/v) (2X), followed by liquid-liquid partitioning and modified CB silica column cleanup. Deoxynivalenol is quantitated by gas chromatography of the trimethylsilyl derivative. Initial recovery studies with pure deoxynivalenol show 70 to 75% recovery at the 5 ppm level. This method also quantitatively extracts zearalenone from field-inoculated corn. Samples of infected corn and feeds were tested successfully for zearalenone levels in a study to determine the effect of *Fusaria* spp. on pregnancy in swine. The study, done in cooperation with Drs. John Tuite and Gerald Long, Purdue University, indicated the *Fusarium roseum* toxins presented early fetal development.

e. Specific Objective: Deoxynivalenol for standards and biological studies will be prepared from field-inoculated corn or *in vitro* fermented substrates.

Progress: Deoxynivalenol was isolated from laboratory-inoculated substrates (Fusaria isolates) and purified by high-pressure liquid chromatography. Characterization of the new trichothecene reported last year was completed; the structure is 3,15-dihydroxy-12,13-epoxy-trichothec-9-ene-8-one. Corn, barley, wheat, and rice were tested at three different temperatures for deoxynivalenol production by Fusaria isolated from ear and stalk-rot damaged corn. Initial results show production levels by the isolate-inoculated corn from 0-120 ppm in 21 days.

f. Specific Objective: Continue study for methods and transmission of aflatoxins in animal tissues.

Progress: An international collaborative study on the NRRC method for determining aflatoxins in animal tissues was conducted. The method was submitted to the Association of Official Analytical Chemists and the International Union of Pure and Applied Chemistry with the recommendation for adoption as an official method. In a cooperative study with A. C. Pier and J. L. Richard, ARS National Animal Disease Laboratory, three 1200-pound Holstein cows were given oral doses of aflatoxin $B_1$. Two cows were given 0.35 mg/kg $B_1$/kg cow per day for 3 days; cow 1 was sacrificed 24 hours after the last dose and cow 2, 6 days after the last dose. Sixteen tissues plus milk, blood, urine, rumen content, and fecal samples were tested for aflatoxin.

Aflatoxins $B_1$ and $M_1$ were detected in all samples except thymus of cow 1 and aflatoxin $B_2$ (0.02-0.35 ng/g) was present in many tissues. The highest levels of $B_1$ and $M_1$ were found in the liver (7.1 ng $B_1$/g and 6.1 ng $M_1$/g) and kidney (1.3 ng $B_1$/g and 56.6 ng/g) of cow 1 which confirms last year's results obtained on a 300-pound steer. Aflatoxins $B_1$ and $M_1$ (0.02-1.5 ng/g) were found only in the liver, kidney, and urine of cow 2, indicating that aflatoxins are eliminated from cows' tissues. When contaminated feed is withdrawn, urine could be monitored to determine when tissues are aflatoxin-free to the point that the cattle are marketable.

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3. Metabolites of Toxin-Producing Fungi Found in Corn and Other Cereal Grains (M. D. Grove)

a. Specific Objective: Elucidate reaction pathways of intermediates formed during ammoniation of an aflatoxin-like model compound.

Progress: The 3,5-dimethoxyphenol formed by ammonia-induced decomposition of an aflatoxin-model ketocoumarin undergoes further oxidative ammoniation when the reaction is carried out on a solid matrix that provides a large surface area such as Celite or corn. Several colored products are formed, all in very low yield. One of these was isolated and identified as 1,7,9-trimethoxy-3-phenoxazone. This bright orange compound is also produced when the ketocoumarin is ammoniated on Celite. The molecular weight 206 phenol produced during high temperature and pressure ammoniation of aflatoxin $B_1$ could form an analogous phenoxazone under conditions used for ammoniation of corn.

b. Specific Objective: Initiate studies on the detoxification of ochratoxin A.

Progress: Based on a report in the Danish literature that ochratoxin A levels in contaminated barley could be significantly reduced by ammoniation, preliminary experiments in this area were conducted. Treatment of ochratoxin A with ammonium hydroxide at 70°C for 7 days followed by chromatographic examination of the mixture failed to produce any evidence of degradation.

c. Specific Objective: Determine the reactivity of the dihydrofuran portion of aflatoxin under conditions used for ammoniation of corn.

Progress: Total synthesis of 6-methoxy-3a,8a-dihydrofurobenzofuran in sufficient quantities for testing has been precluded by low yields of intermediates and the lack of a successful reaction of the final step in the synthesis. Yields of intermediates have been improved, and evidence for the final elimination reaction has been observed in mass spectrometric analyses.

d. Specific Objective: Investigate production of toxic metabolites by Penicillium viridicatum.

Progress: Fermentation conditions and isolation techniques were developed for production of the yellow metabolite, viridicatumtoxin, which is produced by a South African strain. Isolation of the pure metabolite from partially refined culture extracts was readily accomplished by a single preparative high-performance liquid chromatographic step followed by recrystallization. Over 3 g of viridicatumtoxin has been furnished by Dr. W. Carlton, Purdue University, who is conducting toxicological evaluations. An HPLC method was developed to quantitate viridicatumtoxin in culture extracts. An HPLC method for the simultaneous quantitative analysis of xanthomegnin, viomellein, ochratoxin A, and citrinin has also been developed.

e. Specific Objective: Study Fusarium strains for their ability to produce metabolites that cause animal feeds to be unpalatable.

Progress: One Fusarium moniliforme strain that produces, on a corn medium, an extractable substance that is refused when added to the drinking water of mice (feed refusal) has been selected for intensive study. This strain produces several antibiotics. One of the antibiotics has been selected for further study, and methods for its production, partial purification, and microbial and TLC assays have been established. A few milligrams of this compound have been purified for use as a TLC standard.

f. Specific Objective: Identify secondary metabolites synthesized by Penicillia, Fusaria, and other common fungi from cereals.

Progress: Fusarium poae NRRL 3287, F. nivale NRRL 3289, and F. moniliforme NRRL 3197, each grown on cracked corn (13 days at 28°C), produced trichothecenes. T-2 toxin (30 μg/g) was detected in corn fermented with F. poae, vomitoxin (1 μg/g) by F. nivale and T-2 toxin (33 μg/g) and vomitoxin (1.5 μg/g) by F. moniliforme. The latter strain was shown to be a cultural variant of the species F. tricinctum (Cda.) Sacc. The indoles penitrem A and B and roquefortine were isolated from cultures of Penicillium cyclopium NRRL 6093. The yield of roquefortine was 148 mg/l. Elaboration of the neurotoxin roquefortine concurrent with the tremorgenic penitrems suggests a potential hazard of this mold because it is commonly found in stored grains.

g. Specific Objective: Assess toxicoses of farm animals thought to be caused by consumption of moldy grains or feeds made from infected grains.

Progress: Several samples of feed or corn thought to be involved in mycotoxicoses of swine, chickens, cattle, and horses were evaluated for toxic principles. Three corn samples, which swine refused to eat, were found to contain vomitoxin. Two chicken-feed samples were evaluated for moniliformin; none was found. However, extracts of one of the samples was found to be lethal to mice and produce skin irritation on the back of a rabbit. The extract was examined for the presence of 12 trichothecenes, and none were found. Two mass spectral computer programs have been written: one to produce mass chromatograms to highlight the mass number associated with trichothecenes, and the second to perform a general search for other compounds of potential interest.

h. Specific Objective: Continue collaborative studies with the University of Illinois, College of Veterinary Medicine, of mycotoxicoses in equine.

Progress: Strains of Fusarium moniliforme isolated from corn judged to be involved in a field case of equine leucoencephalomalacia (ELEM) were cultured on corn at 25°C. When the moldy corn was fed to donkeys, it failed to produce ELEM. However, many of the corn samples cultured with these F. moniliforme strains were toxic to day-old ducklings and caused death within 3 days.

i. Specific Objective: Study biological activity and fate of trichothecene metabolites.

Progress: Methods are being developed for analysis of vomitoxin (supplied by NRRC) in urine and plasma at the Department of Veterinary Biosciences, University of Illinois. Recoveries are >70% at 0.1 μg/g. For gas chromatographic analysis, vomitoxin is derivatized with heptafluorobutyrylimidazole and detected by electron capture. In a study of the acute toxicity of vomitoxin in broiler chickens carried out in collaboration with the Department of Poultry Science, North Carolina State University, the oral $LD_{50}$ of vomitoxin was estimated to be 140 mg/kg. Acute vomitoxicosis produced extensive hemorrhaging characteristic of the hemorrhagic anemia syndrome. In a study with the University of Guelph, Ontario, swine were fed moldy corn containing vomitoxin. A 2.9 μg/g level of vomitoxin did not appear to influence feed intake, while a level of 5.8 μg/g of feed significantly reduced feed intake.

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4. Origin and Ecology of Mycotoxin-Producing Fungi in Grain (D. T. Wicklow)

   a. Specific Objective: Determine the mechanism(s) by which Aspergillus niger prevents aflatoxin contamination when sharing a substrate with A. flavus.

      Progress: Experiments have shown that A. niger interferes with A. flavus ability to produce aflatoxin on autoclaved corn both by lowering substrate pH (<4.0) and through the production of a water-soluble metabolite(s). In related experiments where aflatoxin $B_1$ was artificially introduced onto autoclaved corn kernels, A. niger neither degraded the aflatoxin nor converted it to aflatoxin $B_{2_a}$.

   b. Specific Objective: Compare the principal mycotoxins (aflatoxins and major indole metabolites) in sclerotia of A. flavus strains with those in sclerotia of A. parasiticus strains to determine their significance in distinguishing isolates at the species level.

      Progress: Isolates of A. flavus from both cool and warm latitudes were cultured on potato dextrose agar containing yeast extract to identify sclerotia-producing strains. Chloroform-methanol extracts of sclerotia were analyzed for the presence of aflatoxins and major indole metabolites (e.g., cyclopiazonic acid, aflatrem, and dihydroxyaflavinine). Aflatoxin is reported from sclerotia of A. flavus for the first time. Cyclopiazonic acid was detected primarily in sclerotia of isolates from warmer latitudes. Aflatrem and dihydroxyaflavinine were detected in sclerotia from 85% of the strains examined. These metabolites are associated with the sclerotial stage of the life cycle, because neither were detected in extracts of the culture medium and mycelium of Petri dish cultures from which all the sclerotia were removed. Geographic variation and intrafungal allocation of these toxic compounds in A. flavus are examined from the evolutionary ecologist's perspective of selective forces shaping the chemical defense systems of fungi.

   c. Begin studies on the role of the sclerotium in the life cycle of A. flavus group (A. flavus, A. parasiticus) with particular reference to agricultural (corn) systems.

      Progress: Sclerotia of A. flavus were produced during the moist chamber incubation of kernels from corn that was either naturally infested in the field or artificially inoculated in a plant growth room. Large numbers of sclerotia were also produced when A. flavus-inoculated kernels were incubated on nonsterile garden soil. Because A. flavus-infested kernels commonly reach the soil surface in the form of downed ears or through spillage at harvest, sclerotia may represent an important source of A. flavus inoculum in field soils where corn is grown.

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5. Microbial Species Interactions and Development of Aflatoxin in Preharvest Corn (Cooperative Agreement - University of Wisconsin)

   a. Specific Objective: Contrast aflatoxin accumulation in Biotron-grown corn under a climate regimen typical of the upper midwest (aflatoxin rare) vs. southeastern (aflatoxin common).

Progress: Regionally popular commercial corn hybrids, two planted in the upper midwest Corn Belt and two grown in the southeastern states, were successfully grown to maturity (dried down; approximately 15% kernel moisture) in a specially modified plant growth room (Biotron, University of Wisconsin). Ears were inoculated with A. flavus conidial suspension at 7, 14, and 21 days following emergence of silks. Inoculation consisted of mist spraying exposed silks or the toothpick wounding (through husk) to simulate insect damage. Silk-inoculated hybrids from the upper midwest showed little aflatoxin ($B_1$ = <4 ppb/ear). Wounding of ears promoted aflatoxin development. Substantial quantities of aflatoxins were detected in the wounded kernels themselves. Furthermore, 52 intact kernels, positioned adjacent to 18 toothpick-wounded kernels on a single ear, averaged 5900 ppb $B_1$/ear. Aflatoxin levels in kernels from the southeastern hybrids are presently being determined.

b. Specific Objective: Determine the extent to which common molds isolated from corn, including A. flavus, are able to invade and spread to individual kernels when inoculum was introduced by spraying silks or through mechanical wounding of individual kernels.

Progress: A. flavus, A. niger, Fusarium moniliforme, and Penicillium funiculosum are capable of spreading from toothpick-wounded kernels, where they were introduced as inoculum, at either 14 or 21 days following silk, to adjacent intact kernels. Significantly, only F. moniliforme and P. funiculosum were able to invade adjacent kernels when conidial inoculum was applied at 7 days post-silk. These data provide evidence that stage of kernel maturation is a critical factor in determining whether individual mycotoxin-producing fungi are capable of kernel infestation. Experiments now in progress are aimed at measuring the effects of specific fungal interactions on A. flavus invasion and aflatoxin contamination of preharvest corn.

6. Application of the Trickle Ammonia Process to Drying Corn at Ambient Temperatures (Cooperative Agreement - University of Minnesota)

a. Specific Objective: Initiate project on determining optimal conditions for on-farm and elevator use of trickle ammonia process for drying corn at ambient temperatures to conserve energy.

Progress: One bin of high-moisture (25-26%) corn has been treated with ammonia and dried at ambient temperatures with a circulating fan. When the temperature dropped to freezing, the fan was turned off. In the spring, the fan will be turned on. A bin of control corn was dried using heat. Both will be studied microbiologically and tested for mycotoxins. Ammonia in the test bin is being measured by litmus paper and adsorption on silica gel cartridges. The odor of ammonia was not an indication of measurable ammonia.

7. Mycotoxins in Food and Feedstuffs (P. L. 480 Grant - Pakistan Council of Scientific and Industrial Research Laboratories, Lahore)

a. Specific Objective: Survey aflatoxin content of samples of wheat, rice, corn, and milk, and of feed ingredients such as peanut press cakes, cottonseed cakes, mustard seed cake, and soybean meal. Evaluate rapid aflatoxin detection methods on these crops.

Progress: This project began in February 1981. Fifty samples of food and feed ingredients have been examined and no aflatoxin found in 20 wheat samples. However, seven feed samples were positive with the highest level in corn gluten (800 μg/kg). The other positive samples were dried bread, sunflower cake, rice polishings, and mixed poultry feed.

HORTICULTURAL AND SPECIAL CROPS LABORATORY

L. H. Princen, Chief

Research Leaders: R. Kleiman, J. A. Rothfus, C. R. Smith, Jr., and H. L. Tookey

## A. BREEDING AND PRODUCTION OF FORAGE CROPS FOR HAY, PASTURES AND OTHER USES INCLUDING TURF

1. Chemicals in Tall Fescue Affecting Livestock Health and Forage Utilization (S. G. Yates)

   a. Specific Objective: Separate, identify, and where possible, quantitate low-level constituents identified in toxic subfractions from fescue.

      Progress: A GLC method of analysis to allow a rapid comparison of the profile of organic acids in toxic forage to non-toxic forage has been devised. In addition to the organic acids previously reported, 2,4-dihydroxybutyric acid is twofold higher in a toxic anion fraction than in a nontoxic fraction. Toxic hays fractionate into approximately 80% fibrous residue, 1% anions, <1% alkaloids and cations; lipids and neutrals constitute the remainder.

   b. Specific Objective: Prepare fractions of toxic hays for testing in cattle.

      Progress: Two large-scale extracts of hay (ca 220 lb) were prepared for testing in cattle: (1) highly toxic KY-31 was extracted and separated into anion, alkaloid, and alkaloid-free cation fractions; and (2) lowperloline variety 307 was fractionated also into similar components. The KY-31 hay contained 47 mg/g formyl loline alkaloid whereas the 307 hay contained 296 mg/g formyl alkaloid. Perloline content is not known at this time. Results in cattle are shown under 20100-002A.

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      YATES, S. G. Tall Fescue Pasture Toxins. In Handbook of Naturally Occurring Food Toxicants, M. Rechcigl, ed., CRC Press, Boca Raton, FL, in press.

2. Bioassay of Chemical Constituents of Tall Fescue Forage (Cooperative Agreement - University of Missouri)

   a. Specific Objective: Examine possible small animal assays and evaluate via bovine IP assay fractions or mixtures that promise exceptional potency.

Progress: Rats were given anion fraction A that lowered foot temperature of the cow. Blood pressure measurements in the rat under conditions of relaxed arterioles (high-ambient temperature) failed to give consistent results. Summer syndrome was investigated by IP injection of suspect fractions into cattle held under stress of humidity and temperature (78-82°F). Frequent urination, slobbering, and reduced feed intake were taken as clinical signs. Such signs were seen from the administration of alkaloid-free cations particularly KY-31, but the alkaloids or the anion of G1-307 variety of tall fescue showed no significant effect.

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## B. INTRODUCTION, CLASSIFICATION, MAINTENANCE, EVALUATION AND DOCUMENTATION OF PLANT GERMPLASM

### 1. Chemical Analysis of Uncultivated Plants (R. Kleiman)

a. Specific Objective: Chemically screen seeds and characterize novel constituents in seed oils and other plant components.

Progress: More than 100 new species were analyzed for oil and protein content. The extracted oils were examined by gas and thin-layer chromatography and by IR and UV spectroscopy. Oil content as high as 67% (*Sacoglottis gabonensis*, from Ghana) and nitrogen content as high as 7.5% (*Croton* sp., from China) were found. Seed oils from many were judged unusual from TLC and GC analyses. Fatty acid composition was determined from oils of 227 different species. Significant amounts of unusual fatty acids were found in the following:

| Species | Family | Unusual component |
|---|---|---|
| *Lomatia silaifolia* | Proteaceae | 25% 16:1 |
| *Telopea mongaensis* | Proteaceae | 31% 16:1 |
| *Petrophila canescens* | Proteaceae | 17% 16:1 |
| *Baliospermum montanum* | Euphorbiaceae | 83% conj. 18:3 |
| *Momordica grosvenori* | Cucurbitaceae | 24% conj. 18:3 |
| *Impatiens pallida* | Balsaminaceae | 52% conj. 18:4 51% oil |
| *Pittosporum phillyreoides* | Pittosporaceae | 23% 20:1, 9% 22:1 |
| *Reaumuria hypericioides* | Tamaricaceae | 7% ricinoleic |
| *Dialyanthera gordoniaefolia* | Myristicaceae | 19% 12:0, 49% 14:0 |
| *Sphaerolobium vimineum* | Leguminosae | 3% 3:0, 9% 10:0, 6% 12:0, 8% 14:0 |
| *Martinezia lindeniana* | Palmae | 57% 12:0, 24% 14:0, 55% oil |
| *Pinus flexilis* | Pinaceae | 15% 5,9,12-18:3 |

| | | |
|---|---|---|
| Juniperus commonis | Cupressaceae | 18% 5,11,14-20:3, 4% 5,11,14,17-20:4 |
| Litsea cubeba | Lauraceae | 83% 12:0, 8% 10:0 |
| Reinwardtia indica | Linaceae | 70% epoxy oleic |

Ricinoleic acid has never been reported in the Tamaricaceae nor has epoxyoleic acid been reported in the Linaceae. Characterization of the fatty acids of Grevillea decora seed oil resulted in the discovery of four new hydroxy acids, 7-hydroxy-cis-17-docosenoic acid, 9-hydroxy-cis-19-tetracosenoic acid, 11-hydroxy-cis-21-hexacosenoic acid, and 13-hydroxy-cis-23-octacosenoic acid. These long chain acids, totalling about 15% of the fatty acids, coexist with omega-5 non-oxygenated acids from $C_{14}$ to $C_{28}$ in the seed oil. The triterpene esters of Dolichothele longimamma were identified and quantified. Fatty acids, $C_8$ to $C_{18}$, were found esterified to the C-3 hydroxyl groups of beta-amyrin, methyl oleanolate, maniladiol, erythrodiol, and longispinogenin. Cooperation with Japanese workers resulted in the discovery of new sterols and components which can distinguish between virgin and B-residue olive oils. Thirty-one Meliaceae species were analyzed by glass-capillary GC for cis-vaccenic content: One genus, Entandrophragma, contained as much as 50% vaccenic acid.

b. Specific Objective: Analysis of potentially new crops in cooperation with plant breeders.

Progress: Over 100 samples of crambe seed were analyzed in cooperation with the Agricultural Research Center, Beltsville, MD, for fatty acids and oil content. In general, both erucic acid and oil content were lower than desired. Seed from 51 Chinese Tallow (Sapium sebiferum) trees were analyzed to evaluate U.S. germplasm. Analysis included tallow, kernel oil, fatty acids of both, and the estolide content of kernel oil. Significant variation existed in all components analyzed. Ninety samples of rape-seed were examined for fatty acid and glucosinolate content in cooperation with Washington State University and University of Idaho. Four additional rapeseed samples were analyzed for cooperators. Three were low-glucosinolate selections, containing 0.7 to 0.8% glucosinolate, and the fourth sample contained a "normal" level at 4.8% glucosinolate. Erucic acid content of the latter was 44.5%, while that of the low glucosinolate selections ranged from 47 to 55%. In the oil, increasing erucic acid content was associated with increasing linolenic (18:3) and decreasing 18:1, 18:2, and 20:1 acids. Fatty acid analysis was accomplished on 5 new species of Cuphea, source of medium-chain fatty acids. Two were rich in $C_{10:0}$, two in $C_{14:0}$, and one in $C_{18:2}$.

c. Specific Objective: Develop mass spectrometric methods for the analysis of plants and plant related materials.

Progress: Chemical ionization-mass spectrometry (CI-MS), using several reagent gases, was applied to fatty acids, wax-esters and polar lipids. In CI-MS of fatty acids, the amount of fragmentation varies from only quasimolecular ion to considerable fragmentation in CI spectra of polyhydroxy fatty esters. However, unlike EI spectra, CI spectra of polyfunctional fatty esters do show ions indicative of molecular

weight. CI of wax-esters provide spectra which make calculation of the fatty alcohol and acid combinations far less complicated than from EI spectra. CI of phosphatidylcholines provides both molecular weight and acyl moiety information. An HPLC procedure was developed, using an IR detector, which quantitates the estolide triglycerides present in Sapium seed oils. Fatty acid methyl esters from 2 to 3 Cuphea seeds were analyzed by first extraction in pentane after homogenizing the sample and subsequent analysis by both packed and capillary GC.

d. Specific Objective: Evaluate agronomics of Vernonia galamensis in the United States.

Progress: Vernonia galamensis seed was provided to informal cooperators in Maryland, Kentucky, Iowa, New Mexico, and Oregon. Small plots were planted at Corvallis, OR, Murray, KY, and Peoria, IL. Plants reached heights of 6 feet in Corvallis and Murray but failed to blossom. In Peoria, limited data from fertilizer trials suggest some response of Vernonia to applied nutrient levels. No blossoms formed on growth chamber grown plants under extended lighting (18 hr days). Whole plants were collected in the Peoria trials for possible future analyses.

e. Specific Objective: Develop means of forming superior coatings from epoxy oils.

Progress: Mono-, di-, and trivernolin triglycerides have been isolated from vernonia oil by column chromatography. The oxirane content of vernonia oil has been doubled by chemical epoxidation, and epoxy fractions have been obtained chromatographically from this material.

f. Specific Objective: Assist in commercialization of crambe and continue cooperation with Murray State University on seed banking and demonstration plots for crambe.

Progress: Unexpected aphid infestations destroyed ca. 80% of the 1,000 acres of crambe contracted privately by a company seeking domestic sources of high erucic acid oils. About 50 acres was salvaged for seed purposes through our cooperation with Murray State University. Additional acreage (for planting seed) was contracted privately in New Mexico in August, and grower meetings are planned or currently taking place to initiate an expanded commercial effort in 1982.

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PLATTNER, R. D., R. E. ENGLAND, K. L. PAYNE-WAHL, AND S. G. YATES. Falcarinol in Carrots by GC-MS. American Society for Mass Spectrometry, Minneapolis, MN, May 24-29, 1981.

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2. Biologically Active Plant Constituents for Pest Control and Medicine (C. R. Smith, Jr.)

a. Specific Objective: Detect biological activity in extracts of seed and other plant parts through both in-house screening and outside cooperators.

Progress: During the past year, 80 new extracts have been prepared, submitted to National Cancer Institute contractors, and are in varying stages of bioassay. Of these, 3 have shown preliminary anti-leukemic (PS) activity, not yet confirmed. For pesticidal activity, one new extract has been bioassayed in-house with European corn borer (Ostrinia nubialis Hübner) larvae as well as fractions derived from Dipoclisia glaucenscens (Menispermaceceae) and Apium selowianum.

b. Specific Objective: Isolate and characterize active constituents from plant extracts with confirmed biological activities.

A total of eight biologically active maytansinoids have been isolated from Trewia nudiflora seed; the structure of three of these has been

reported previously. Three additional maytansinoids have been fully characterized, and these represent a new class of maytansinoids with two fused macrocyclic rings; in addition to the usual 19-membered ring, there is a 12-membered ring which is a novel feature. In addition to potent antitumor activity in experimental systems, these maytansinoids exhibit several pest control activities. These include antifeedant activity towards European corn borer (Ostrinia nubialis) larvae and the spotted cucumber beetle (Diabrotica undecimpunctata); morphogenic effects on the codling moth (Laspeyresia pomonella); also disrupts life cycle of redbanded leaf roller (Argyotaenia velutinana and reduces progeny in the plum curculio (Conotrachelus nenuphar). In addition, these compounds are toxic to the striped cucumber beetle (Aclymma vittatum) and the chicken body louse (Menacanthus stramineus). (Most of the foregoing insect bioassays were carried out by Dr. David Reed, ARS, Vincennes, IN.) The ethanol extract from 20 kg of Diarthron vesiculosum (Thymeleaceae) seed has been concentrated and fractionated extensively by solvent partitioning. After the ethanol is partitioned between water and methylene dichloride, the antitumor activity resides in the latter solvent; material from methylene chloride has been fractionated extensively by column chromatography and considerable enrichment of activity has been achieved.

Work on the bioactive principle of Sesbania drummondii has been resumed, and a new compound has been isolated which is difficult to separate from sesbanine (characterized previously) but is structurally unrelated to sesbanine. Elucidation of the new structure is underway.

Ethanol extraction of 45 kg of Diploclisia glaucescens (Menispermaceae) seed yielded an ethanol extract, portions of which have been fractionated extensively by solvent partitioning, preparative HPLC, and preparative TLC. About 100 g of the principal insectidal alkaloid (provisionally named diploclisin) has been isolated, and it appears to be a rather polar $C_{27}$-$C_{28}$ steroidal alkaloid bearing at least four hydroxyl groups and an $\alpha,\beta$-unsaturated ketone grouping. Diploclisin is accompanied by small amounts of three other compounds, probably closely related; these four are exceedingly difficult to separate from each other, although progress is being made by alternating TLC and HPLC.

An additional 75 kg of Thevetia thevetioides seed has been extracted and fractionated to provide 54 g additional neriifolin for entomological studies by E. C. Berry, ARS, Ankeny, IA; Dr. David Reed, ARS, Vincennes, IN; and others that may become interested.

c. Specific Objective: Identify constituents of oat extracts that attract the saw-toothed grain beetle (Oryzaephilus surinamensis L.).

Progress: The nonvolatile constituents of rolled oat extracts have been fractionated and bioassayed. Mixtures of free fatty acids are the only fraction producing consistent attractancy in the saw-toothed grain beetle. Considerable variation has been found in the composition of volatiles from oats of various origins. Oat volatiles have been separated into various classes of compounds; bioassays indicate attractancy resides in hydrocarbon, carbonyl, and alcohol fractions.

Among carbonyl compounds identified are: hexanal, heptanal, octanal, nonanal, benzaldehyde, and 2,4-nonadienol acetate; some of these show attractancy, but a strong, consistent response is not observed. Hydrocarbons identified include: Toluene, styrene, and xylenes; these are inactive.

d. Specific Objective: Identify constituents from peach wood extracts that attract the female peach borer moth.

Progress: The emphasis has been shifted from wood to bark. Fractionation of peach bark extracts has culminated in identification of four compounds: styrene (inactive), benzaldehyde (moderately active), methyl and ethyl benzoate (both moderately active). Our collaborator, Dr. Reed, has just found one fraction which is both attractive and an oviposition stimulant.

e. Specific Objective: Identify the resistance factors of pest-resistant apples.

Progress: Several varieties of Golden Delicious apples, picked at various intervals during the growing season and shipped to us by Dr. H. Goonewardene (Purdue Univ.), have been sequentially extracted by a series of solvents of increasing polarity. Dr. Goonewardene has found that ethanol extracts significantly deter the feeding of apple maggots, thus opening the way for fractionation to isolate active substances.

f. Specific Objective: Determine chemical basis for difference between varieties of corn which differ in their resistance to the rice weevil (Sitophilus oryzae).

Progress: Sequential extractions have been carried out with different varieties of corn furnished by Dr. J. G. Rodriguez, U. of Kentucky. The resulting extracts have been bioassayed by Luis Gomez, Dr. Rodriguez graduate student; attractancy was found to reside in the ethanol extract. Fractionation to pinpoint active compounds now becomes possible.

g. Specific Objective: Determination of triglyceride structure of Trewia nudiflora seed oil.

Progress: Trewia nudiflora seed oil, which accumulated in gallon quantities during our extraction of the seed for other purposes, was known from previous work to contain substantial concentrations of kamlolenic (18-hydroxyoctadeca-cis-9, trans-11, trans-13-trienoic) acid. The neat, untreated oil was found to be nearly impossible to investigate structurally because of its propensity for polymerizing. Accordingly, an alternative procedure was developed as follows: the oil is expressed while seeds are submerged in hexane; the resulting hexane solution is filtered and hydrogenated catalytically at ambient temperature and pressure. The resulting hard, waxy, crystalline product is stable and is amenable to TLC and other manipulations. Results indicate a large number (at least 12) chromatographically

distinct components, most of which have estolide structures and contain up to six fatty acid units per triglyceride molecule. Some of the estolide chains are terminated by methyl groups and others by hydroxyl groups.

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3. Major Fatty Acids from Indian Seed Oils and Their Possible Industrial Use (P. L. 480 Grant - Aligarh Muslim University)

a. Specific Objective: Screening Indian flora for unusual and potentially useful lipid constituents; synthesis of potentially useful derivatives.

Progress: Among the classes of fatty acid derivatives synthesized during the past year are: tetrahydrofurans, oxazoles, and enol acetates. In addition, a new hydroxy acid, isoricinoleic acid, has been isolated and characterized. Extracts of Solanum indicum, S. khasiana, and Heliotropum supinum have been identified as pest-controlling plant materials.

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## C. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

1. Plant Cell and Tissue Culture for the Bioproduction of Valuable Chemicals (N. E. Delfel)

   a. Specific Objective: Investigate the regeneration of whole plants from cultured cells.

      Progress: Of the two means of reproducing plants from culture, organogenesis and embryogenesis, the latter method gives more normal plants. In a series of experiments, Chenopodium quinoa and Cephalotaxus harringtonia have been induced to form globular embryoids, the first step in embryogenesis. If further development can be stimulated, it will be the first time either genus has been regenerated, and the first reproduction of a woody gymnosperm (C. harringtonia) by this means.

   b. Specific Objective: Determine the locus of secondary metabolism in Cephalotaxus harringtonia trees, and relate findings to cultured cells.

      Progress: Incubation of C. harringtonia leaf homogenates or acetone leaf powders either with amino acid precursors of alkaloid biosynthesis (PHE and TYR), or with certain possible phenolic intermediates, did not increase the alkaloid levels. Apparently, the correct intermediates were not supplied, and the biosynthetic pathway for cephalotaxine and its derivatives will have to be determined before extending these experiments.

   c. Specific Objective: Explain lack of alkaloid-ester production by non-producing trees.

      Progress: C. harringtonia trees which were discovered not to produce alkaloid esters were growing at the Northern limit of their range, suggesting environmental control of biosynthesis. Seedlings from these trees were grown in an environmental growth chamber at 25°C and 8, 12, or 16 hr daylength or on a window ledge with a southern exposure, together with alkaloid-esters producing seedlings from other sources. None of 20 seedlings from the non-producer trees contained alkaloid-esters under any of these conditions, whereas the producer plants contained the high levels found previously. This proves conclusively that non-production is genetically rather than environmentally controlled.

d. Specific Objective: Study the uptake and storage of secondary products in culture.

Progress: Cephalotaxus harringtonia cells growing on media containing 6.67 µg/ml of the alkaloid cephalotaxine or its ester isoharringtonine took up both from the medium. With cephalotaxine 1.12 µg/g fresh weight was found in the tissues together with 0.45 µg/g of a second compound tentatively identified as acetyl cephalotaxine from its GLC retention time. With isoharringtonine, 0.36 µg of isoharringtonine, 0.05 µg cephalotaxine and 0.11 µg of acetyl cephalotaxine per gram were present. Neither fed alkaloid was converted to the other alkaloid esters normally found in the tree. Unrelated alkaloids were also transported and stored by the tissues: brucine (33.3 µg/ml fed, 0.9 µg/g FW found), and nalorphine (33.3 µg/ml fed, 1.6 µg/g FW found).

Callus from non-alkaloid ester producing trees was green and embryogenic, that from producers was dark brown and undifferentiated. Suspension cultures from these two cultures were qualitatively different also. The dense brown cells of the latter variety persisted in suspension, and were reminiscent of the "resin" cells implicated in secondary metabolite storage in other plants. This raises the possibility of their separation via density-gradient centrifugation or other means and further culture and characterization.

e. Specific Objective: Study polyamine effect on cell growth.

Progress: Polyamines, such as spermine, stimulate a large number of processes of replication, transcription, and translation essential to growth and multiplication of the cell. Spermine was added to the medium of C. guinoa cells at 0.2 mM, 1 mM, and 5 mM to look for effects on cell growth and differentiation (normal physiological concentration approximately 1 mM). All concentrations of spermine inhibited growth (FW and DW) of C. guinoa and no sign of differentiation was observed. Inhibition may be non-specific under the culture conditions tested since a 5 mM $NH_4H_2PO_4$ control also suppressed growth, suggesting elevated N-levels may be a factor (1 mM spermine = 4 mM N).

Reports:

DELFEL, N. E. Uptake and Metabolism of Alkaloids and Related Phenolic Compounds by Cephalotaxus harringtonia Callus Cultures. Presented at the Annual Meeting of the American Society of Plant Physiologists and the Canadian Society of Plant Physiologists at Laval University, Ste.-Foy, Quebec, Canada, June 14-18, 1981 and at the Gordon Research Conference on Plant Cell and Tissue Culture, Andover, N.H., June 22-26, 1981.

Abstract. Plant Physiol. Suppl. 67(4) (1981):143.

2. Photosynthetic Pigments and Primary Photoacts for Increasing Efficiency and Yield of Crops (J. A. Rothfus)

a. Specific Objective: Continue study of photosynthetic rate in soybean mutants and etiolated bean leaves as it relates to the number of photosystem I and II particles present and their fluorescence emission spectra.

Progress: Further examination of low temperature fluorescence in green and yellow soybeans for evidence of chlorophyll segregation into specific photosynthetic apparatus demonstrated that 740 fluorescence, which was previously correlated with pigment content in several genotypes, is an artifact of self absorption and is thus unsuitable for photosystem II measurements in leaf discs. Similarly, less than satisfactory measures of photosystem II were obtained with the diphenyl carbazide-dichlorophenol indophenol couple. Direct measures of the primary electron acceptor, Q, as absorbance change at 320 nm provided more accurate assessments of photosystem II activity. Determination of absorption cross sections of photosystems I and II and their relative levels in green soybeans and in Clark $Y_{11}$ yellow soybeans show that there is more Q per chlorophyll molecule in green soybeans than in the yellow mutants. Contrary to certain experience of others, photosystems I and II are approximately twice as large in green soybeans as in the yellow mutant variety. The lower levels of photosystem II in mutant plants are consistent with the absence of grana stacking in such plants and with their reduced photosynthetic capacity.

b. Specific Objective: Investigate possible presence of a chlorophyll a/c protein complex in the Phaeodactylum tricornutum LHPP particle as well as possible energy transfer from chlorophyll c to a in organisms and particles. Characterize protein subunits of the complex with regard to size and ionic charge.

Progress: The light harvesting pigment protein complex of P. tricornutum was resolved into a yellow and two green fractions. Elements of the yellow fraction have gel exclusion molecular weights of about 70,000 daltons, but treatment with thioethanol in 1% sodium dodecyl sulfate reduces the molecular weight to ca. 12,000. Amino acid analyses yield an empirical molecular weight of 20,000, which suggests the yellow elements are hexamers of two different subunit proteins. The elements of both green fractions have lower apparent molecular weights than yellow elements and those of the first green fraction appear much larger than those of the second green fraction. Based on areas of HPLC profiles, the concentrations of fucoxanthin, chlorophyll a and carotene are significantly higher in the yellow fraction than in either green fraction whereas concentrations of chlorophyll c's and chlorophilide a are much greater in the green fractions than in the yellow.

c. Specific Objective: Study effect of type, substrate, temperature, and environment on energy transfer from auxiliary pigments to chlorophyll in model systems formed from adsorbed molecules.

Progress: Methods were established for the absorption of chlorophyll a and other pigments on hydrophyllic surfaces such as glass or sugars and on hydrophobic surfaces formed from treated glass or reverse-phase HPLC packings, and techniques were developed for obtaining fluorescence and reflectance spectra of these adsorbed layers. It thus appears possible to chemisorb chlorophyll a onto glass using chlorophyllase from Nitzschia to break down chlorophyll in an acetone-water buffer in the presence of the glass substrate. Adsorption alters spectral properties of the pigments. Reflectance peaks of fucoxanthin adsorbed on reverse-phase HPLC packing are shifted toward longer wavelengths compared to the same pigment on sugar. Similar spectral shifts are apparent with fucoxanthin dissolved in carbon disulfide compared to solutions in methanol, but fucoxanthin in hexane does not show the same shift. Pigment extracts from Nitzschia closterium adsorbed on a hydrophobic substrate exhibit fluorescence peaks at 465 nm and 510 nm that could be caused by energy transfer. These peaks are not present in spectra of the unadsorbed or desorbed extract. A relatively weak peak at 465 nm appears in excitation spectra of fucoxanthin-chlorophyll a mixtures on the same hydrophobic substrate. Comparisons of spectra for adsorbed extracts and adsorbed fucoxanthin-chlorophyll a mixtures indicate that energy transfer in the adsorbed extracts Nitzschia extracts cannot be due to chlorophyll a-fucoxanthin interaction alone.

d. Specific Objective: Continue work on the effects of bicarbonate on photosystem II reactions (cooperation with A. Stemler, University of California, Davis, CA).

Progress: An effect of bicarbonate was sought to account for previous observations by others that bicarbonate-deficient chloroplasts show 30 to 50% inhibition of Hill reaction in low-intensity light. With broken chloroplasts from peas, the decay of chlorophyll a fluorescence yield in the millisecond and seconds range following a single flash was multiphasic with a very slow component of one to two seconds half time. This slow component, which is enhanced two or threefold in bicarbonate-depleted chloroplasts, corresponds to inhibition of the $Q^-B \rightarrow QB^-$ reaction. Consistent with this proposed site of action, a 40% inhibition of oxygen flash yield in bicarbonate-depleted chloroplasts is relieved to a great extent by reducing the excitation flash rate from 2 to 0.33 Hz. The single flash results and flash rate dependence differentiate this bicarbonate effect from other less-significant effects on other electron transferring photoreactions. The maximum amplitude of variable fluorescence yield and 520 nm absorption change after a single flash are unaffected by bicarbonate depletion, and the dark distribution of oxygen-evolution S-states is shifted to a more reduced configuration in depleted chloroplasts. Thus normal charge separation occurs in bicarbonate-depleted photosystem II reaction centers, but a large fraction of $Q^-$ decays so slowly that in effect a portion of the photosystem II centers remain closed to photochemistry.

e. Specific Objective: Investigate effects of divalent cations on photosystem II.

Progress: The apparent ability of magnesium ion to enhance chloroplast oxygen evolution was examined in cooperative studies with R. Khanna (Smithsonian Institution, Rockville, MD). Measurements of oxygen evolution with a concentration electrode in saturating light show no significant effect of magnesium supplement on divalent cation-deficient chloroplasts. Magnesium enhancement seen with a rate electrode can be duplicated by allowing magnesium-deficient chloroplasts to settle onto the oxygen electrode surface. Conditions that preclude such settling reduce oxygen flash yields by an order of magnitude. These results, which demonstrate a significant effect of magnesium on the boyant density of chloroplasts correct interpretations of earlier workers, who had concluded that divalent cations alter the number of active photosystem II reaction centers.

f. Specific Objective: Continue work with corn genotypes and correlation of yield with photosynthetic efficiency.

Progress: Five corn genotypes representative of commercial varieties introduced at approximately 10-year intervals since 1930 were analyzed during development for chloroplast pigments. At the same time, microscopic examinations of chloroplasts from the same plants at 40 days assessed morphological differences between the various genotypes. Sampling four leaves in descending alternating positions showed that two varieties maintained constant levels of chlorophyll a in all leaves while the other varieties had high chlorophyll concentrations in old leaves. Correlations between leaf concentrations of chlorophyll a or accessory pigments and yield were not obvious. Pigment ratios, however, did not vary markedly with leaf age, and could thus be averaged for a composite representative of whole plant pigment levels for each genotype. Thus, xanthophyll/carotene ratios increased with yield while chlorphyll a/b ratios decreased with yield. Such changes are consistent with enhanced grana formation, and, indeed, microscopic analysis indicated that bundle sheath stacking correlated fairly well to genotype and yield. Genotypes from the 30's and 40's were essentially devoid of bundle sheath stacking in all leaves whereas samples from the 60's and 70's exhibited considerable stacking that involved as many as five thylakoids per granum. The pigment data and morphological changes suggest that the higher-yielding corn varieties possess elevated levels of photosystem II activity relative to that of photosystem I. Such results would not be inconsistent with the recent proposal by Arnon and coworkers [Proc. Natl. Acad. Sci. 1981, 78(5) 2942-2946] that these two photosystems are essentially autonomous.

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ESKINS, K. AND L. HARRIS. High-Performance Chromatography of Etioplast Pigments in Red Kidney Bean Leaves. Photochem. Photobiol. 33(1) (1981):131-133.

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ESKINS, K., W. F. KWOLEK, AND L. HARRIS. The Accumulation of Accessory Pigments as a Function of Chlorophyll a. A Comparison of Development and Genetic Control. Physiologia Plantarum. In press.

GARDNER, H. W. AND P. A. JURSINIC. Degradation of Linoleic Acid Hydroperoxides by a Cysteine·$FeCl_3$ Catalyst as a Model for Similar Biochemical Reactions. I. Study of Oxygen Requirement, Catalyst and Effect of pH. Biochim. Biophys. Acta 665 (1981):100-112.

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Other Reports:

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JURSINIC, P. A. AND A. STEMLER. An Explanation for the Low Light Intensity Bicarbonate Effect. Presented at Annual Meeting American Society for Photobiology, Williamsburg, VA, June 14-19, 1981.

JURSINIC, P. A. New Measurements on Double Advancement in S-States of Oxygen Evolution and on Double Turnovers in Photosystem II Charge Separation. Presentation to DOE Plant Research Laboratory, Michigan State University, East Lansing, MI, May 20-22, 1981.

## D. TECHNOLOGIES FOR FOOD AND FEED USES FOR FIELD CROPS

1. Composition and Properties of Seed Lipids for Foods and Feeds (J. A. Rothfus)

a. Specific Objective: Characterize thermal properties of polyunsaturated acids and related esters and triglycerides.

Progress: Isomeric unsaturated acids available through intra-Center cooperation (cf. Oilseed Crops Laboratory, D.1.) were characterized via differential scanning calorimetry. Four geometric isomers of 9,10-dideuterio-12,15-octadecadienoic acid and the corresponding methyl esters exhibit small entropic differences that might arise from different end group packings. Entropies of fusion suggest a higher degree of order in ester crystals than in the corresponding acids even though the esters melt at lower temperatures. The 12-cis,15-trans compounds are most stable entropically yet they melt lower than their 12-trans,15-cis counterparts and the 12-trans,15-trans compounds, which are highest melting. These results disclose a subtle effect of unsaturation on thermal properties and imply that structure-function relationships can be identified for lipids. An apparent end group packing effect is even more obvious with deuterated methyl linoleate. The nondeuterated ester and two tetra-deuterio isomers (15,15,16,16-deuterio and 16,16,17,17-deuterio) all behave rather similarly, but the 17,17,18,18-deuterio isomer melts at a lower temperature with less enthalpy and entropy change. This terminally deuterated compound, unlike the other isomers, also crystallizes imperfectly if cooled rapidly and undergoes an exothermic reorganization when heated from the supercooled fictive state. Comparable analyses of the corresponding acids, which are yet to be prepared, is expected to yield a more precise description of the presumed end-group effect.

The thermal properties of saturated single acid triglycerides of acid chain lengths $C_8$ through $C_{30}$ show consistencies that emphasize the role of specific molecular transformations in determining solid-state properties of lipids. However, characteristics typical of specific structures; e.g., odd or even chain length, may persist only throughout discrete moelcular weight ranges. Changes in melting point patterns, particularly for $\beta'$ and $\beta$-forms, correlate with a fundamental change in chain length effect at $C_{14}$. Similarly, phase excitation energies for $\alpha$-form transformations show odd-even alternation for short chainlengths but increase linearly with chain length above $C_{14}$, which evidences the importance of extended chain conformation and lateral chain interactions as determinants of polymorphic properties of the longer chain molecules. In triglycerides, as in n-alkanes, this controlling effect of the extended chain apparently predominates when mid-chain interactions contribute 60 percent or more of the total

molecular interaction. When extrapolated to longer chain lengths, α-phase excitation energies exceed measured heats of fusion for higher-melting (β' and β) forms. In effect, the energy required to excite low-melting forms of large triglycerides may be sufficient to disrupt all higher-melting forms. This could account for the common absence of β' or β-forms from simple thermal scans of saturated triglycerides $C_{24}$ and above.

b. Specific Objective: Correlate Raman spectral variations of single acid triglyceride polymorphs with their crystal structure transformations.

Progress: Two β'-polymorphs of tristearin distinguished by distinctly different Raman spectra were further characterized by X-ray diffraction analysis. Patterns for the two forms are nearly identical, but that for $\beta_1$'-tristearin is sharper, indicating greater crystallinity, and it contains two distinct diffraction lines at 4.1 and 4.3 Å. The diffraction spectrum for $\beta_2$'-tristearin contains a single line at 4.2 Å and duplicates spectra reported for β'-tristearin by earlier workers. Low angle X-ray diffraction studies indicate the β'-cell structures arise from significant change in the angle of chain tilt, which would also result in alteration of lateral chain interactions. Better understanding of the transient polymorphic options available to tristearin is being pursued through current Raman studies on the forces or structural features that contribute to apparent β'-form stability in odd-chain length triglycerides.

c. Specific Objective: Continue computer modeling of triglyceride structures.

Progress: Molecular interaction energies computed by summation of intermolecular atomic interactions for 54 different combinations (nine arrangements of molecules each involving six different molecular conformations) of α-form triglycerides demonstrate that for a given set of circumstances several molecular combinations can have essentially equivalent stabilities. Thus differences in the routes to such states may be as important as molecular configuration in determining the physical properties of solid lipids. Similarly, interaction energies computed for 72 different combinations of odd or even chain length β'-form triglycerides are, thus far, not drastically different from those of α-form molecules. However, β' configurations that produce nonparallel rows of extended zig-zag chains appear less stable than parallel configurations, which would favor further conversion to β-form configurations. This result is consistent with the behavior of even chain length triglycerides, which readily convert to high-melting β-forms, but as yet the computational models make no clear distinction between even-chain length triglycerides and odd-chain triglycerides, which tend to melt from β'-forms. Comparisons of X-ray data with dimensions from space-filling models and agreement between observed entropies of fusion and entropies calculated from conformational probabilities emphasize the importance of extended chain conformation and they suggest conformational differences and possibly different β' to β conversion pathways for odd versus even chain length triglycerides.

Entropy calculations that agree with α-form heats of fusion suggest that only two of the three aliphatic chains in a triglyceride need activation to achieve α-form melting. Such correlations to real data provide valuable constraints in selecting from among configurations and conversion pathways judged equally plausible on the basis of computed interaction energies.

d. Specific Objective: Investigate admixture perturbations of known triglyceride thermal properties.

Progress: Differential scanning calorimetry was used to examine the effects of a known phospholipid impurity on the polymorphic properties of known triglycerides. Results suggest an unusual concentration-dependent and perhaps time-dependent, stabilization of low melting forms of a single-acid saturated triglyceride, but not those of a mixed-acid triglyceride. These observations, if corroborated by additional studies, provide new insight into mechanisms by which solid fat properties might be regulated.

2. Soybean Analysis for Improved Quality (R. Kleiman)

a. Specific Objective: Determine oil and protein contents of soybean samples in order to develop improved varieties.

Progress: About 15,700 samples were received from public soybean breeders throughout the United States and Canada. These samples were examined for their oil and protein content by the infrared reflectance method, and the data were reported to the cooperating breeders.

b. Specific Objective: Provide fatty acid composition of selected soybean samples in order to lower the linolenic acid content through plant breeding.

Progress: Fatty acid composition was determined on 8,000 soybean samples. Included in these gas chromatographic analyses were 2,500 germplasm collection samples from the northern and southern collections. These analyses complete the fatty acid profile of the southern collection. Analysis of 5,500 chemical mutation samples from J. Wilcox, ARS, Purdue University, yielded several low linolenic acid samples, one as low as 3.4%.

c. Specific Objective: Develop rapid analytical procedures for soy protein in order to raise the methionine and cystine content through breeding.

Progress: TLC separation procedures using Chromarode followed by analysis on an Iatroscan was evaluated. No solvent system was found which gave adequate separation in the silica gel rods.

Reports:

The Uniform Soybean Tests, Northern States, Compiled by J. R. Wilcox and A. D. Knapp, West Lafayette, IN. 1980.

Evaluation of Soybean Germplasm II, Compiled by E. E. Hartwig and C. J. Edwards, Stoneville, MS. 1980.

KLEIMAN, R. AND J. F. CAVINS. Analysis for Improved Soybean Quality. 1981 Annual Report of the American Soybean Association, pp. 75-76, St. Louis, MO. 1981.

## E. RECLAMATION AND REVEGETATION OF LAND AREAS DISTURBED BY MAN

### 1. Trace Element Uptake and Distribution in Agricultural Crops Grown on Disturbed Lands (K. D. Carlson)

a. Specific Objective: Evaluate effect of sewage sludge application to stripmined land on the growth of nonfood crops, notably crambe and kenaf.

Progress: Crambe and kenaf were grown the third year with the same treatments used in 1979 and 1980 [control (C), fertilizer (CF), 50 ton/acre sludge ($S_{50}$), 100 ton/acre sludge ($S_{100}$)]. A post emergence herbicide was effective in controlling barnyard grass on the sludge plots, although a wet spring and late planting date (June 1) combined to create potentially the worst infestation to date. Both crops were adversely affected by the grass until it was brought under control. Four sets of 10 randomly selected crambe plants were harvested from each plot on August 25 (C, CF) and September 11 ($S_{50}$, $S_{100}$), and three subplots (each 0.001 acre) from each kenaf plot were harvested on November 23. At harvest, the uniform stand of kenaf on all plots averaged ca 60,000 plants/acre and reached maximum heights of 8 feet. Preliminary data from only one of three replicates suggests that 1981 dry matter yields of kenaf will be uniform and somewhat lower (2-2.5 ton/acre) than 1980 yields where plant densities were up to four times higher. Crambe plants from 1980 and 1981 harvests are still being prepared for analyses.

b. Specific Objective: Determine uptake by these nonfood crops of heavy metals that may be contained in sewage sludge.

Progress: Gross soil, sludge, and commercial fertilizer samples from 1979-1981 have been prepared by quartering and grinding techniques for metal and other analyses. Kenaf samples from the 1981 harvest will soon be ready to join those from 1979-1980 for metal analyses. While 1979 crambe plant material is ready for metal analyses, 1980 and 1981 material will not be ready before mid-1982 for analyses. Early soil analyses indicate that significant decreases in pH are occurring in some but not all plots. Electrical conductance of sludge-treated soils increased after a single treatment (1980 vs. 1979), and dramatic increases (three- to sixfold) in exchangeable sodium occurred for all treatments the first year. Moderate or small decreases in exchangeable calcium and magnesium also occurred. Initial organic content of the soil was low (<0.5%) as was available phosphorus (nil).

Reports:

CARLSON, K. D. AND R. L. CUNNINGHAM. Progress Report, Field 24. Big Bluestem Advisory Committee, Spoon River College, Canton, IL, May 8, 1981.

CARLSON, K. D. AND R. L. CUNNINGHAM. Progress Report, Field 24. Big Bluestem Advisory Committee, Spoon River College, Canton, IL, August 14, 1981.

CARLSON, K. D. AND R. L. CUNNINGHAM. Report to the Big Bluestem Advisory Committee, Spoon River College, Canton, IL, November 6, 1981.

CUNNINGHAM, R. L. AND K. D. CARLSON. Progress Report on Crambe and Kenaf on Test Plots in Field 24. Big Bluestem Advisory Committee Meeting, Spoon River College, Canton, IL, February 5, 1981.

## F. UTILIZE, MANAGE, AND CONSERVE SOIL FERTILITY FOR INCREASED PRODUCTION AND NUTRITIONAL QUALITY OF PLANTS AND ANIMALS

1. Improve and Implement the Determination of Isotopic Nitrogen in Soil Samples (R. Kleiman)

   a. Specific Objective: Cooperate with ARS soil scientists in determining amount of $^{15}N$ in samples developed through nitrogen X tillage experiments.

   Progress: Procedures were established in which cooperating scientists provide NRRC with dry $NH_4Cl$ samples that are derived from soil and plant materials. The samples are now shipped in vials that fit directly into the automatic sampler developed for the nitrogen isotope mass spectrometer.

   Samples were received from cooperators in Illinois, Nebraska, and Kentucky. The samples, totaling approximately 2500, were analyzed for atom $\%^{15}N$ and the results returned to the respective scientists.

   b. Specific Objective: Develop automatic procedure to convert $NH_4Cl$ to $N_2$ and introduce it into the mass spectrometer for analysis of atom %N.

   Progress: An automated system to generate nitrogen gas from $NH_4Cl$ and transfer the gas directly to the mass spectrometer has been completed and has functioned now for several months without serious breakdown. The system utilizes automated air activated valves to control gas introduction and liquid nitrogen cooling. A computer-controlled relay system was put on-line which activates all systems external to the mass spectrometer, including the sampler. The sampler holds 36 samples which allows automatic operation for over 70 samples per day.

Report:

RAYFORD, W. E., R. KLEIMAN, AND R. D. PLATTNER. Automated Nitrogen Isotope Ratio Analysis. American Society for Mass Spectrometry, Minneapolis, MN, May 22-29, 1981. Proc. Annu. Conf. on Mass Spec. and Appl. Topics, p. 252 (1981).

## G. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Natural Toxicants in Horticultural Crops and Cruciferous Feeds (H. L. Tookey)

   a. Specific Objective: Evaluate biological activity of selected glucosinolate products that occur in significant amounts in cruciferous vegetables.

   Progress: Goitrin at 30 mg/kg/day (4X) increases rat liver weight and urinary ascorbic acid output; it increases pentobarbital sleeping time in males but not in females. Several nitriles that are hydrolytic products of glucosinolates that occur in significant amounts in crucifer vegetables have been isolated in pure form from seed sources in amounts sufficient for preliminary toxicological evaluation: 1-cyano-2-hydroxy-3-butene, 1-cyano-3,4-epithiobutane, 1-cyano-4-methylsulfonylbutane, and 1-cyano-3-methylsulfinylpropane. Phenylpropionitrile was supplied from a commercial source.

   These have been sent to the ARS Western Regional Research Center to be tested both as direct toxicants and as teratogens. Falcarindiol is more toxic than falcarinol. A further test is needed to report on $LD_{50}$.

   b. Specific Objective: Discover convenient sources of natural toxicants needed for future testing.

   Progress: Several species containing glucosinolates of interest have been planted to increase seed stocks. Berteroa incana (containing 2-hydroxy-4-pentenyl glucosinolate) was planted at Ames, Iowa, and recently harvested, but yield data are not yet available; Eruca sativa (4-methylthiobutyl-GS) was planted (2/5 acre) last fall in Corvallis, Oregon, because spring-planted Eruca did not grow well or produce seed; Arabis turrita (unknown GS, probably novel) was spring planted but none seeded. Arabis will be replanted in the greenhouse for spring transplant. Isatis aucheri var. vellifera (unknown GS also occurring in turnip) also did poorly; available seed for replanting is low (40 seeds).

   c. Specific Objective: Provide data base for evaluation of newly developed cultivars in regard to their glucosinolate content.

   Progress: Samples of Oriental crucifer vegetables have been extracted preparatory to analysis. These were grown in Wisconsin by Dr. P. H.

Williams and include Japanese radish (23 cultivars), Korean radish (27), Chinese greens (7), and Chinese flowering kale (6). This assortment does not quite complete the morphological range of common Oriental crucifer vegetables. Domestic cauliflower (3 cultivars) and turnips (11) are also included.

d. Specific Objective: Carry out survey of falcarinol, falcarindiol, and myristicin in market carrots.

Progress: Samples of nine carrot cultivars grown at Madison, Wisconsin, contain 0-2 ppm myristicin, 1-21 ppm falcarinol, and 38-268 ppm falcarindiol. One parsnip cultivar contains a high level of myristicin (290 ppm), but less falcarinol (51 ppm) and falcarindiol (124 ppm). The four major commercial varieties, Spartan Bonus, Danvers 126, Hi-color 9, Imperator 58, are being grown in five states (California, Arizona, Florida, Wisconsin, and Illinois) to determine year-to-year and location-to-location variation in toxicants. Analyses from 1981 crops generally fall in the same ranges as did the Wisconsin cultivars cited above.

e. Specific Objective: Study the mechanism of formation of toxic epithioalkylnitriles from glucosinolates.

Progress: The mechanism for the formation of ω-epithioalkylcyanides is the same in a number of Cruciferae genera. Thioglucosidase from Brassica, Crambe, Armoracia, or Sinapis interacts with epithiospecifier protein from Brassica or Crambe.

f. Specific Objective: Cooperate with the ARS Eastern Regional Pasture Laboratory, University Park, PA, in evaluating Lathyrus sylvestris as a forage.

Progress: Results from the 1980 crop year do not show a simple relationship between harvest date and 2,4-diaminobutyric acid. The unknown component, presumably an amino acid, has not yet been satisfactorily identified.

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CARLSON, D. G., M. E. DAXENBICHLER, C. H. VANETTEN, H. L. TOOKEY, AND P. H. WILLIAMS. Glucosinolates of Crucifer Vegetables: Turnips and Rutabagas. J. Agric. Food Chem. 29 (1981):1235-1239.

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2. Cattle Feeding Tests of Crambe Meal as a Protein Concentrate (Cooperative Agreement - Purdue University)

   a. Specific Objective: Obtain Food and Drug Administration clearance for using crambe meal in beef cattle rations.

      Progress: FDA announced final rule on permitting use of crambe meal, heat toasted, in feed of beef animals; published in the Federal Register 46(108):30081-30082 (June 5, 1981). Consequently, the CRIS Unit has been terminated. This development may make the cultivation of crambe for its high-erucic oil economically viable because the byproduct meal can be sold as a feedstuff.

3. Inheritance of Glucosinolates in Crucifer Vegetables (Cooperative Agreement - University of Wisconsin)

   a. Specific Objective: Gain information on the inheritance of specific glucosinolates found in cruciferous vegetables.

      Progress: A rapid-cycling stock of turnip (genome aa·r) low in goitrin is being developed. Brassica nigra (bb) has reached the fourth selection generation (M4); B. juncea (aabb) has reached M4; B. napus (aacc) has reached M2 and has dwarfing incorporated; B. carinata (bbcc) and Raphanus sativa have reached M3. B. campestris (aa) has both high and low glucosinolate stocks at the second backcross generation. A rapid-cycling stock of B. oleracea (cc) was initiated using Chinese flowering kale and cauliflower.

Report:

WILLIAMS, P., C. HILL, AND H. LEUNG. Rapid Cycling Stocks as an Aid to Genetics and Breeding of Crucifers. Presented at Crucifer Improvement Conference, Madison, Wisconsin, August 31-September 1, 1981.

4. Biological Effects of Potential Toxicants from Glucosinolates (Cooperative Agreement - Colorado State University)

   a. Specific Objective: Gain information on the sequence of lesion development in rat livers as a result of feeding 1-cyano-2-hydroxy-3,4-epithiobutanes.

      Progress: The cooperative agreement was activated in July 1981. Preliminary tests on dispersion of 1-cyano-2-hydroxy-3,4-epithiobutanes in rodent rations are in progress as are tests of stability of the toxicant in the stored rations. A 90-day feeding study will begin as soon as stability parameters are known. Early histological changes such as alterations in hepatic microvillous membranes will be examined by electron microscopy.

# OILSEED CROPS LABORATORY

T. L. Mounts, Chief

Research Leaders: E. A. Emken, E. N. Frankel, E. H. Pryde, and W. J. Wolf

## A. TECHNOLOGIES FOR FOOD AND FEED USES FOR FIELD CROPS

1. Effects of Vegetable and Animal Trypsin Inhibitors in Long-Term Animal Feeding Studies (Cooperative Agreement - University of Minnesota)

   a. Specific Objective: Complete the first series of long-term feeding tests of soybean trypsin inhibitors.

   Progress: At the University of Minnesota, periodic sacrifice of rats fed 15 different diets, revealed that the first incidence of pancreatic nodular hyperplasia occurs after 15 months of continuous feeding of raw and toasted soy flour diets. The incidence of hyperplasia in high-trypsin inhibitor (TI) and low-TI diets was 80 and 16%, respectively. Rats fed casein diets exhibited 0% incidence. Similar results were obtained with rats sacrificed at 18 months. Dietary protein levels of 10, 20, and 30% appear to have little influence on the TI-induced hyperplasia. Histological examination of pancreata of rats sacrificed at 20, 22, and 24 months is underway.

   At the ARS Western Regional Research Center, sacrifice of rats on a chronic 2-year rat feeding trial with 26 different diets containing raw and heated soy flours, soy protein isolates, and casein, began on September 8, 1981. Survival rate of the Wistar rats was greater than 85% for all test diets. Histopathological examination of all major organs and other selected tissues is underway.

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   LEE, S. S. AND I. E. LIENER. A Non-Invasive Technique for Assessing Pancreatic Function in Rats Fed Raw and Heated Soybean Diets. Nutr. Repts. Int. 24(6) (1981):1173-1178.

   LIENER, I. E. Factors Affecting the Nutritional Quality of Soya Products. J. Am. Oil Chem. Soc. 58(3) (1981):406-415.

2. Improving Food Quality of Soy Oil Products and Their Stability to Heat- and Light-Catalyzed Oxidation (E. N. Frankel)

   a. Specific Objective: Study secondary products from photosensitized oxidized fatty esters.

   Progress: Studies of photosensitized oxidation of methyl linoleate show that the greater relative concentration of 9- and 13-hydroperoxides than 10- and 12-hydroperoxides is characteristic of singlet oxygenation

and not due to simultaneous autoxidation. Cyclization of the internal 10- and 12-hydroperoxides accounts for their lower relative concentrations. Secondary products separated by silicic acid and high-pressure liquid chromatography were characterized spectrally (IR, UV, $^{1}$H-NMR, $^{13}$C-NMR, GC-MS). Major secondary products included diastereomeric pairs of 13-hydroperoxy-10,12-epidioxy-trans-8-octadecenoate (I) and 9-hydroperoxy-10,12-epidioxy-trans-13-octadecenoate (II); minor secondary products included hydroperoxy oxygenated, dihydroperoxides, and epoxy esters.

Thermal decomposition of the hydroperoxy cyclic peroxides produced hexanal and methyl 10-oxo-8-decenoate as major volatiles from I, and methyl 9-oxo-nonanoate and 2-heptenal from II. The mechanism for thermal decomposition involves cleavage of the cyclic peroxide ring and on either side of the hydroperoxide. Hydroperoxy cyclic peroxides are therefore suggested as important flavor precursors in oxidized fats.

An authentic cyclic peroxide hydroperoxide was synthesized as a model epidioxide related to prostaglandin endoperoxides formed in photooxidized methyl linolenate. The methane sulfonate of methyl ricinoleate was reacted with hydrogen peroxide. The resulting homoallylic hydroperoxide was cyclized readily to yield a saturated hydroperoxy-epidioxide and a hydroperoxy-cyclopropane ester in about equal amounts. The homoallylic structure of the peroxy hydroperoxide precursor is an essential feature of the cyclization that is now recognized as an important process in the autoxidation of methyl linolenate and in the photosensitized oxidation of methyl linoleate. The synthetic saturated cyclic peroxide product is a useful model compound that has proved valuable in our structural studies of different prostaglandin-related compounds found in oxidized lipids. These hydroperoxy cyclic peroxides may be of biological importance.

b. Specific Objective: Study mechanism of photooxidation of soybean oil.

Progress: Minor constituents of soybean unsaponifiables were separated from crude oils to evaluate their photocatalytic and inhibiting activity. Methods were developed to separate nonglyceride constituents with and without saponification. Further separations were achieved by column chromatography and by HPLC using both reverse and normal phase systems. Fluorescence methodology was developed to estimate chlorophyll, carotenoids, and tocopherols in crude soybean oils and in their unsaponifiable chromatographic fractions. Manometric photooxidation of methyl linoleate was investigated at 5-10°C to minimize free radical autoxidation. Sterol, lutein, and tocopherol fractions all inhibited photooxidation. Chlorophyll was a strong pro-oxidant. Beta-carotene inhibited initially the photooxidation but promoted it at later stages of the reaction. These basic studies are suggesting that soybean oil processing can be improved by achieving a better balance between singlet oxygen quenchers (photooxidation inhibitors such as carotenoids and tocopherols) and photosensitizers (such as chlorophyll).

c. Specific Objective: Investigate cyclic acids in oils heated under deep-frying conditions.

Progress: To resolve current concern over the potential toxicity of heated oils, cyclic acids were analyzed in commercial samples obtained in this country and in the Middle East. The U.S. samples were derived from local restaurants (just before disposal) and from a hospital cafeteria. The samples from Egypt and Israel were collected by Dr. S. El-Magoli (University of Cairo) and Dr. P. Budowski (Hebrew University) from street vendors frying vegetable patties (a popular Middle Eastern food called "fallafel") in open air stands. The U.S. samples ranged from 0.1 to 0.4% in cyclic acids, and from 2 to 8% polar/non-eluted thermal oxidation materials. The Egyptian and Israeli samples showed significantly more heat abuse with values for cyclic acids ranging from 0.2 to 0.7% and polar materials ranging from 2 to 22%.

For future investigations of biological effects and characterization of heated fats, two new model cyclic fatty methyl esters were synthesized: 10-(6-ethyl-3-cyclohexenyl-9-decenoate and 8-(6-butyl-3-cyclohexenyl)-7-octenoate. With these two new compounds, a selected, homologous series of five, pure diunsaturated $C_{18}$ cyclic esters was thus completed. To aid identification and separation of the new cyclic esters from the normal fatty esters found in used, deep-frying oils, their retention characteristics were determined and compared by capillary GLC. The new compounds were characterized by spectral methods and gas chromatography-mass spectrometry. Several cis and trans geometric isomers also separated and identified. A large-scale preparation of methyl 9-(6-propyl-3-cyclohexenyl)-8-nonenoate was achieved for feeding studies with laboratory animals in collaboration with Dr. E. G. Perkins, University of Illinois.

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3. Nutritional Quality, Safety, and Flavor Aspects of Soybean Protein Products (J. J. Rackis)

   a. Specific Objective: Investigate the possibility of separating and recovering trypsin inhibitors (TI) from a potato juice concentrate (PJC) using ultrafiltration and other techniques (Cooperative with the Eastern and the Western Regional Research Centers).

      Progress: A number of procedures were attempted to separate the non-inhibitor type proteins from the trypsin inhibitors in PJC. These involved heat coagulation, ultrafiltration (UF), and acid precipitation. A major loss of TI activity was experienced with heat coagulation.

The separation obtained with ultrafiltration was unsatisfactory. Non-inhibitor type proteins were precipitated with acid at pH 3.5 leaving the TI in solution. In a subsequent UF separation, low MW impurities were removed in the permeate leaving a retentate fraction in which the TI had been concentrated 7-8 fold. All of the PJC was processed by this procedure to yield 15.4 kg of freeze-dried retentate which has a TI activity of 270 mg/g.

b. Specific Objective: Complete studies of cyanide analysis in soy products.

Progress: Cyanide content was determined on distillates from soybean meal, various soybean fractions and soybean products by a pyridine-barbituric acid colorimetric procedure. Values of 0.05-0.3 μg CN/g of sample in soy protein products and 1.24 μg/g in soybean hulls were obtained when browning was kept to a minimum. These results compare with values as high as 1-3 mg/g in cassava and certain varieties of lima beans, respectively, and with values of 0.001-0.45 μg/g reported in various cereal grains and cereal products. Much higher, more variable results resulted under severe browning to charring conditions. Interferences with analyses were investigated and accounted for. Emulsin and a crude linamarase preparation were ineffective in releasing cyanide from soybean meal. Extracts of soybean meal were fractionated by column chromatography and tested for cyanide. No cyanogenic precursor was isolated and little concentration of cyanide was obtained. These studies indicated that cyanogenic compounds are present in very small amounts in soybean protein products and most likely are of little nutritional significance.

c. Specific Objective: Complete characterization of the soy peroxidases; determine whether the peroxidases can catalyze the oxidation of soy phenolics; and determine whether peroxidase-generated oxidation products can cooxidize polyunsaturated lipids as a source of objectionable flavors in soy protein products.

Progress: The methodology and conditions needed to separate soybean peroxidases via gel isoelectric focusing were determined with recently purchased electrophoresis equipment. Isoelectric focusing of soybean peroxidases on both polyacrylamide and agarose gel matrices gave three major bands of activity with isoelectric points estimated to be 4.2, 4.0, and 3.9. Phenyl borate matrix has no selectivity for separating soybean peroxidase isozymes based on results from testing a variety of different eluents prescribed by the manufacturers of the gel. Poor recovery of peroxidase activity, i.e., 60%, eliminates usefulness of this column material in our purification scheme.

Model systems were developed that demonstrate ability of soybean peroxidase to cooxidize polyunsaturated fatty acids (PUFA). Our initial efforts involved use of indoleacetic acid for the oxidative reaction. However, with this substrate, $H_2O_2$ was required to initiate the reaction. We found that soybean peroxidase will oxidize the reduced form of β-nicotinamide adenine dinucleotide (NADH) by molecular oxygen with no $H_2O_2$ as catalyst. Action of peroxidases on NADH is

known to generate superoxide anion radicals. Cooxidation of PUFA initiated with either biochemically generated superoxide anion radicals or chemically generated superoxide ions via action of phenazine methosulfate on NADH under aerobic conditions both failed. However, we found that addition of $Fe^{+3}$ to the model system will cause a rapid oxygen uptake with PUFA but not with alcohol or ester forms of these acids. Iron will complex with the carboxylic acid group of PUFA. Role of this ligand in the cooxidation reaction is currently being investigated. Based on the stoichiometry of NADH oxidation catalyzed with peroxidase and molecular oxygen we propose:

$$6NADH + 2O_2 \rightarrow 6NAD^+ + 2H_2O + H_2O_2$$

Insolubility of soy phenolics in aqueous buffers preclude their use as hydrogen donors in the peroxidatic reaction.

d. Specific Objective: Determine the kinetics of oxidation and subsequent decomposition of various molecular species of soy phosphatidylcholine (PC).

Progress: Isotopically labeled PC has been synthesized and autoxidized. PC labeled in the choline methyl groups has been used to measure the rate of oxidation of soy PC and preliminary results indicate a rate of oxidation when measured by the amount of radioactivity in oxidized PC to be approximately twice that measured by 234 nm increase. Di 18:2 and di 18:3 PC were synthesized with isotopically labeled fatty acids and autoxidized. Analysis of the oxidized fatty acids from di 18:2 PC shows all radioactive fractions have 234 nm absorbance but a similar analysis of di 18:3 PC oxidized acids shows radioactive fractions which have no ultraviolet absorbance and others with no 234 nm absorbance.

e. Specific Objective: Prepare research protocol for Biochemical Effects of Soy Protein Isolates in long-term rat feeding studies.

Progress: This project is a collaborative effort between NRRC and the Western Regional Research Center (WRRC). The protocols and Standard Operating Procedures (SOP's) for the rat feeding phase WRRC Project Code: Pan #6 and the preparation of soy isolates and formulation of diets NRRC Project Code NRRC-01 are in the developmental process. Three soy protein isolates have been prepared for this study: (a) Heated low-TI soy protein isolate with 3 mg TI/g and high protein digestibility, (b) raw, low-TI soy protein isolate with 5.7 mg TI/g and low protein digestibility, and (c) raw, high-TI soy protein isolate with 30 mg TI/g and poor digestibility. Casein is the control diet. The following analytical data have been acquired on the protein products: moisture, ash, protein, TI activity, nitrite, nitrosamine, and bacterial and mold counts. All products are Salmonella-free. Effect of 6-month storage of the soy isolates and casein on moisture and protein content, TI activity, and microbial counts have been completed. Eight diets, sufficient for 40 rats per diet, were prepared. The rat experiments were initiated on August 4, 1981.

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BAKER, E. C. Development of a Pilot-Plant Process for the Preparation of a Trypsin Inhibitor Rich Fraction from Potatoes. Presented at the 181st National Meeting of the American Chemical Society, Atlanta, GA, March 31, 1981.

4. Analytical and Structure Studies of Soybean Proteins (W. J. Wolf)

a. Specific Objective: Investigate the possibility of using immunological techniques for determining the amount of soy added to food products.

Progress: Buffers containing protein denaturants have been used to extract native and denatured soybean proteins. Buffer solutions containing urea or sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (RSH) proved to be good solvents. Buffered solutions containing Triton X-100 (a non-ionic detergent) were less effective. Procedures were developed to identify both acidic and basic subunits of glycinin (11S) by isoelectrofocusing on polyacrylamide gels containing SDS. Samples of native and denatured soybean proteins have been solubilized in phosphate buffer containing urea-RSH and alkylated. The protein solutions were forwarded to Purdue University for immunological assay.

b. Specific Objective: Develop high-performance liquid chromatography (HPLC) of soybean proteins and evaluate as a rapid analytical technique for determining composition of soybean protein mixtures.

Progress: Soy proteins were prepared fresh by established techniques. Separation of these proteins was marginal on a Waters I-250 protein column, but was satisfactory on TSK Gel 2000 SW and a TSK 3000 SW columns. The HPLC patterns are being compared and correlated with ultracentrifugal analysis.

c. Specific Objective: Quantitate the isoflavone-coumestrol content of soy protein products.

Progress: A high-performance liquid chromatography procedure was developed for the quantitative determination of isoflavones and isoflavone glucosides in soybeans and soybean products. Dehulled, defatted soybean flours contain the following mean isoflavone content (mg/100 g): diadzin 61.7, glycitein-7-β-O-glucoside 12.9, genistin 119.8, daidzein 32.8, genistein 26.7. The same isoflavones were found in soybean protein concentrates and isolates but in decreased amounts. Effects of environment and variety on the isoflavone and isoflavone glucoside contents of soybeans were also studied. Extracting the oil from soybeans did not remove the isoflavones or the isoflavone glucosides. The isoflavone content of soybeans is variable and depends on variety, location, and year grown. On an equal weight basis, most of the

isoflavones were found to be concentrated in the hypocotyl of the soybean. The isoflavone content of pure soybean hull was low.

d. Specific Objective: Purify β-conglycinin and study effect of sonication on its aggregation properties.

Progress: β-Conglycinin has been prepared by published procedures and its purity checked by ultracentrifugation, gel filtration, and gel electrophoresis. β-Conglycinin of 80% purity showed at least 3, 4, and 5 major peaks on chromatography, respectively with Sepharose 2B, Sepharose 6B, and Sephadex G-100. Ultracentrifugally pure 7S fraction is obtainable in 30% yield by successive gel filtration with Sephadex G100 and Sepharose 6B. Sepharose 2B is suitable for separating aggregates produced by sonication and heat-treatment from other proteins. Sonicating water extracts of defatted soybean flakes for 8 minutes produced aggregates of 40-50S species from 7S proteins amounting to 20-40% of the total proteins. The aggregates are stable once they are exposed to buffer of high ionic strength (0.5μ), show maximum stability at pH 6.5-6.8, and resemble those aggregates formed by heating a water extract at 80°C for 10 minutes except that their size is smaller and their rate of formation is slower.

e. Specific Objective: Purify and characterize the soybean protein acid-sensitive fraction to provide information regarding its relationship to soy protein product flavor and functionality.

Progress: A 28% saturated $(NH_4)_2$ $SO_4$-precipitated protein fraction from water-extracted soy proteins was used for preparation of acid-sensitive proteins. Comparison of sodium dodecyl sulfate polyacrylamide gel electropherograms obtained from the parent $(NH_4)_2$ $SO_4$ fraction, the acid-sensitive fraction prepared from it, and the remainder fraction, revealed a band, unique to the acid-sensitive fraction, having an apparent molecular weight of 116,000. The other proteins were present in each of the fractions examined. Urease activity was detected and found to be associated primarily with the acid-sensitive fraction.

f. Specific Objective: Establish the presence or absence of stachyose in wheat flour, devise analytical procedure for determining stachyose in the presence of glucofructans and apply to quantitation of soy in wheat-soy composite flours.

Progress: High-pressure liquid chromatography indicated the presence of little or no stachyose in extracts of U.S. and Bolivian wheat flours, although interferring materials made the results equivocal. Attempts to use galactose oxidase as a specific assay for stachyose (glucofructans that may be present in wheat flour extracts will not react) were unsuccessful because the method was not sensitive enough to measure the low concentrations of apparent stachyose that occur in wheat flour extracts. Work on this objective was therefore discontinued.

g. Specific Objective: Isolation and purification of calmodulin from soybean whey proteins.

Progress: Soybean whey contains 10-30 μg of calmodulin per gram of protein. Soybean calmodulin was prepared by ammonium sulfate fractionation, chromatography with DEAE Biogel A, gel filtration, and affinity chromatography. The purified protein showed a single band on sodium dodecyl sulfate gel electrophoresis and a $Ca^{2+}$ dependency for enzymatic stimulation. Soybean calmodulin may have an affinity for soybean isoflavones because its elution peak coincided with a yellow colored peak in the presence of $Ca^{2+}$, and followed the yellow colored peak in the absence of $Ca^{2+}$. Like calmodulin from beef, soybean calmodulin may have the ability to bind compounds such as phenylthiazine, phenytoin, and pimozide.

h. Specific Objective: Prepare liposomes from soy phosphatidylcholine and determine if lipid oxidation disrupts membrane integrity.

Progress: Liposomes have been prepared from $^{14}C$ labeled phophatidylcholine and large bilayer liposomes were separated from small vesicles by chromatography on Sepharose 4BCL. Preliminary evidence indicates that the liposomes can be fractionated by HPLC which would reduce the separation time from 4 hours to 30 minutes. Liposomes have been prepared in the presence of $^{14}C$ sucrose which resulted in approximately 10% of the sucrose being incorporated inside the liposome. Techniques have been devised to rapidly dialyze the unincorporated sucrose from the liposome preparation.

i. Specific Objective: Survey soybean samples for surface characteristics of their seed coats using scanning electron microscopy.

Progress: Thirty-three soybean cultivars plus a number of plant introductions and strains supplied by Dr. R. L. Bernard, ARS, University of Illinois, were screened using the scanning electron microscope. Pitting of the seed-coat surface occurred in many cultivars, but a number of them were free of pits and degree of pitting varied from light to heavy in those exhibiting this feature. The different cultivars also showed a wide range of a deposit on the seed-coat surface which appeared to be a "fingerprint" of the innermost layer of the seed pod wall.' One cultivar also had deposits of crystal-like materials on its seed coat and the inner surface of the seed pod. Some cultivars exhibited large numbers of cracks through the outer two layers of the seed coat. Intact seed coats were cracked by soaking the seeds in water for a short time and then air drying them; these results support earlier studies suggesting that seed-coat cracking occurs in the field as a result of alternate wetting and drying.

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WOLF, W. J. Present State of Soybean Protein Products in the U.S.A. Presented at Research Institute for Food Science, Kyoto University, Uji, Japan, October 17, 1981, and at meeting of Food and Nutrition Research Association, Tokyo, Japan, October 20, 1981.

5. Supercritical Fluid Technology for the Extraction of Seed Oils (J. P. Friedrich)

   a. Specific Objective: Determine the optimum conditions for extracting soybeans with supercritical $CO_2$ (SC-$CO_2$) and for concurrently separating minor constituents such as free fatty acids, phospholipids, and unsaponifiables; to compare the crude and refined oils obtained by both $CO_2$ and hexane extraction. Also compare the quality of the meal obtained from these extractions.

Progress: Exhaustive extraction of full-fat soybean flakes with SC-$CO_2$ yields an oil that is comparable to hexane-extracted oil except for significantly lower phosphorus content. In a long cylindrical batch extractor, the flakes act much like the stationary phase of a chromatography column, which permits the recovery of light-colored, essentially degummed, crude oil fractions. The solubility of oil in SC-$CO_2$ increased rapidly with increasing pressure. Increasing the temperature increased the solubility at pressures above 6000 psi and decreased it below 6000 psi. Carbon dioxide is an ideal solvent for extraction of food products; it is low in cost, readily available from fermentation processes, and could free a large amount of costly hexane for energy use.

b. Specific Objective: Develop technology required to refine soybean oil extracted with supercritical carbon dioxide.

Progress: Crude soybean oils obtained by extraction with supercritical carbon dioxide (SC-$CO_2$) were processed in the laboratory and compared to hexane extracted oil processed under similar conditions. SC-$CO_2$ extracted oil can be refined with significantly lower refining losses than their hexane extracted counterparts. The lower solubility of free fatty acids and phospholipids in SC-$CO_2$ accounts for the differences. Color removal was similar for both SC-$CO_2$ and hexane extracted oils. Conventional processing (refining, bleaching, and deodorization) produces a pale light colored edible salad oil. Refined, bleached, and deodorized oils from SC-$CO_2$ and hexane processed crudes were evaluated organoleptically. Initially, the oils were of high quality and not significantly different. After accelerated storage at 60°C, the SC-$CO_2$ extracted oils were equivalent to or significantly better than the hexane extracted oils.

Oxidative stability tests showed that refined bleached deodorized oils have similar oxidative stability irrespective of extraction method. However, SC-$CO_2$ extracted crudes are markedly less stable than hexane extracted oils. The absence of phosphatides in SC-$CO_2$ extracted oils accounts for lowered stability, because the same stability was obtained as with hexane-extracted oil when phosphatides were added to SC-$CO_2$ oil.

Publication:

FRIEDRICH, J. P. AND G. R. LIST. Characterization of Soybean Oil Extracted by Supercritical $CO_2$ and Hexane. J. Agric. Food Chem. In press.

Other Reports:

FRIEDRICH, J. P., G. R. LIST, AND A. J. HEAKIN. Petroleum-Free Extraction of Soybeans with Supercritical $CO_2$. Presented AOCS Meeting, New Orleans, LA, May 17-21, 1981.

FRIEDRICH, J. P. Supercritical Fluid Extraction. Presented at NRRC General Staff meeting, Peoria, IL, May 14, 1981.

FRIEDRICH, J. P. Application of Supercritical Fluid Extractions to Cottonseed Processing. Presented at National Cottonseed Products Association meeting, New Orleans, LA, July 20-21, 1981.

FRIEDRICH, J. P. Supercritical Fluid Technology. Presented at ADM, Decatur, IL, September 11, 1981; at VODF-II International Energy Seminar, NRRC, Peoria, IL, October 21, 1981; and Corn Millers' Conference, NRRC, Peoria, IL, June 3, 1981.

Popular articles on our supercritical $CO_2$ extraction work have been published in more than 20 periodicals in agriculture, business, chemistry, feed and industrial technology, including: Chemical and Engineering News, Chemical Marketing Reporter, Business Week, Crops and Soils, Inside R&D, Oil Mill Gazetteer, Journal of Commerce, Biomass Digest, Biotechnical Digest, Delta Farm Press, Feed Stuffs, Southwest Farm Press, SciQuest, Milling and Baking News, Regulatory Watch, Chemical Spotlight, Agricultura de las Americas, Novedades Agropecurias, and the USDA 1981 Yearbook of Agriculture. Additional TV interviews were made on our supercritical fluid research and appeared several times on all three channels in Peoria.

## B. BIOMATERIALS SCIENCE

1. Basic Chemistry of Vegetable Oils for Fuels and Alternative Chemicals (E. H. Pryde)

   a. Specific Objective: Investigate modification of vegetable oils to meet engineering requirements for use in injector pumps of diesel engines.

      Progress: Methyl, isopropyl, and butyl esters of soybean oil were prepared and viscosities determined at 37.8°C (100°F). Viscosities were 4.310, 5.339, and 7.177 centistokes, respectively. The ethyl ester of Pamolyn was prepared and had a viscosity of 5.005 centistokes at 37.8°C (100°F).

   b. Specific Objective: Investigate the possibility of using vegetable oil microemulsions to obtain fuels meeting ASTM properties considered relevant to good engine performance.

      Progress: Aqueous ethanol-soybean oil microemulsions were formulated and their viscosities are near the specified range for #2 diesel fuel. These hybrid fuels are clear, thermodynamically stable liquid fuels, and appear to be micellar systems. Both detergent and detergentless microemulsions were prepared and investigated. Formulations have been developed which may have industrial and agricultural utilization. Currently, engine evaluation tests are in progress at the University of Illinois.

      Publications:

BORUFF, P. A., A. W. SCHWAB, C. E. GOERING, AND E. H. PRYDE. Engine Evaluation of Diesel Fuel-Aqueous Ethanol Microemulsions. Trans. Am. Soc. Agric. Eng. In press.

PRYDE, E. H. Chemical Uses of Vegetable Oil Expected to Increase Sharply. J. Commerce and Commercial, Vol. 347, March 30, 1981, pp. 1A, 6A, and 7A.

PRYDE, E. H. AND H. O. DOTY, JR. World Fats and Oils Situation. In: "New Sources of Fats and Oils," Edited by E. H. Pryde, L. H. Princen, and K. D. Mukherjee, American Oil Chemists' Society, Champaign, Illinois, 1981, Chapter 1.

PRYDE, E. H., L. H. PRINCEN, AND K. D. MUKHERJEE, Editors, "New Sources of Fats and Oils," American Oil Chemists' Society, Champaign, Illinois, 1981.

SCHWAB, A. W., R. S. FATTORE, AND E. H. PRYDE. Diesel Fuel-Aqueous Ethanol Microemulsions. J. Disp. Sci. Tech. In press.

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Other Reports:

GOERING, C. E., A. W. SCHWAB, M. J. DAUGHERTY, E. H. PRYDE, AND A. J. HEAKIN. Fuel Properties of Eleven Vegetable Oils. For presentation at the 1981 Winter meeting of the American Society of Agricultural Engineers. Palmer House, Chicago, IL. December 15-18, 1981.

KAI, Y., AND E. H. PRYDE. Production of Branched Chain Fatty Acids from Sterculia Oil. Paper No. 122 presented at the Evald Skau Memorial Symposium, American Oil Chemists' Society Annual Meeting, New Orleans, LA, May 17-21, 1981.

MILLER, W. R., E. H. PRYDE, AND E. N. FRANKEL. Hydrosilylation of Methyl Oleate. Presented at the Evald Skau Memorial Symposium, American Oil Chemists' Society Annual Meeting, New Orleans, Louisiana, May 17-21, 1981.

PRYDE, E. H. Alternative Chemicals from Vegetable Oils. Presented at the Nebraska Governor's Conference on Utilization of Nebraska Agricultural Products, Lincoln, Nebraska, February 27, 1981.

PRYDE, E. H. AND A. W. SCHWAB. Vegetable Oils as Diesel Fuel: Problems and Possible Solutions. Presented at the 72nd Annual Meeting of the American Oil Chemists Society, May 17-21, 1981, New Orleans, LA, Paper #168.

SCHWAB, A. W. Microemulsion Fuels. AES Cooperator's Conference, October 19-20, 1981, Emerging Technologies in Agriculture, NRRC, Peoria, IL.

SCHWAB, A. W. Preventing Phase Separation in Hybrid Fuels by Emulsification. Presented at Illinois Renewable Fuels Symposium, June 26, 1981, Northern Illinois University, DeKalb, IL.

SCHWAB, A. W., R. S. FATTORE, AND E. H. PRYDE. Diesel Fuel-Aqueous Ethanol Microemulsions. Presented at the 55th Colloid and Surface Science Symposium, American Chemical Society, June 14-17, 1981, Case Western Reserve University, Cleveland, Ohio, Paper #129.

SCHWAB, A. W. AND E. H. PRYDE. Vegetable Oil Microemulsions. Presented at Seminar II (FODF-II), October 21-22, 1981, Peoria, IL.

2. Physical/Chemical Modification of Vegetable Oils for Diesel Fuel (E. H. Pryde)

See Northern Agricultural Energy Center, A.5.

3. Long-Term and Endurance Engine Tests with Vegetable Oil Products as Diesel Fuel (Cooperative Agreement - North Dakota State University)

See Northern Agricultural Energy Center, A.6.

## C. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

1. High-Temperature Soybean Cooking Oils for the Export Market (E. N. Frankel)

a. Specific Objective: Develop basic principles of continuous high pressure hydrogenation as an improved technology for food products in export markets for soybean oil.

Progress: All systems were tested for operation and the reaction with 1% catalyst at 170°C and 1000 psi at an oil flow rate of 1 liter/hr was too slow to be practicable. All further reactions were conducted at 200°C. At an oil flow rate of 1 liter/hr increasing the hydrogen gas flow between 1 to 4 liter/min had no effect on selectivity or activity of the catalyst. Increasing the oil flow increased the reaction rate. These results suggest lack of proper mixing at low oil flow rates. Further experiments are underway to increase mixing in the reactor by injecting hydrogen gas at several points. There is an inherent limitation in the slurry reactor system that does not allow sufficient mixing for proper hydrogenation. However, no major changes in design are contemplated because efforts are being redirected toward a stationary catalyst system with the Berty reactor.

b. Specific Objective: Study the kinetics and mechanisms of Ziegler type catalysts as potential active and selective catalysts for continuous hydrogenation of soybean oil.

Progress: Work was completed and presented in May 1981 at the AOCS meeting in New Orleans. A manuscript has been prepared for publication and submitted to peer reviewers. A patent has been filed on the catalysts.

c. Specific Objective: Develop steam refining process for production of salad-cooking, margarine, and shortening oil.

Progress: Crude soybean oil was degummed in the laboratory and split into two portions. One-half was hydrogenated directly (no bleaching) to two different iodine values: i.e., 90 and 110. The other portion was bleached prior to hydrogenation. After post bleaching the 4 oil samples were steam refined and submitted to organoleptic evaluations. Although some problems were encountered with slight color development, the organoleptic results demonstrated the feasibility of producing a bland edible product by hydrogenation-steam refining techniques.

d. Specific Objective: Evaluate heated oil odor characteristics of processed soybean oil to improve its acceptability in domestic and foreign markets.

Progress: Soybean oil (SBO) and copper hydrogenated soybean oil (CUHSBO) were heated to frying temperature at intervals up to 30 hours and evaluated by a trained panel for odor characteristics. The oils were tested without additives and with citric acid, tertiary butyl hydroquinone (TBHQ), methyl silicone, and an anoxomer (a polymeric antioxidant) in various combinations. The methyl silicone/citric acid combination had the greatest effect of all additives in lowering the odor intensity of both SBO and CUHSBO. The combination of citric acid, methyl silicone, and TBHQ also lowered odor intensity but not as effectively as citric acid and methyl silicone only. The oils containing citric acid only, citric acid and TBHQ, citric acid and an anoxomer and no additives decreased in odor intensity as heating time increased. The effects of the various additives had the widest range of odor scores at 15 hr of heating and tended to equalize at 30 hr.

e. Specific Objective: Conduct sensory evaluations of the individual volatile constituents of soybean oil (SBO) to simulate odors generated during use of soybean oil for cooking and frying.

Progress: Fifteen aldehydes were added singly and in various combinations to heated cottonseed oil (CSO) to determine their contribution to heated SBO quality. The characteristic descriptions of heated SBO include fishy, acrid, and fried food. The individual additions of acrolein, octanal, 2,4-heptadienal, 2,4-octadienal, and 2,4-nonadienal produced acrid odor in CSO and additional odors such as sour and melon. Combinations of aldehydes mostly produced acrid odors. Few descriptions of fried foods were noted and none for fishy.

Soybean oil was evaluated for flavor and GC volatiles profile after accelerated temperature storage. The direct GC method for volatiles showed significant correlations between a consistent pattern of 6 peaks and flavor scores with coefficients ranging from -0.87 to -0.94.

f. Specific Objective: Complete identification of volatiles from oxidized tri(cis-9, cis,15-octadecadienyl) glycerol (isolinolein) and trilinolenin.

Progress: Identified volatiles from oleate hydroperoxides indicate isolinolein would produce eight major unique aldehydes. Data from heated (192°C) isolinolein show 10 primary volatiles are formed. Volatile identification, using our mass spectral library, confirm the

presence of three predicted isolinolein volatiles (ethanol, propanol, and 2-butenal). Mass spectra of 2-pentenal and 6-nonenal, regenerated from their dinitrophenylhydrazones derivatives (received from ERRC), substantiated the presence of two more expected aldehydes. Interpretation of mass spectra of three major isolinolein volatiles indicate the compounds are the anticipated 5-octenal, 2,7-decadienal, and 2,8-undecadienal.

Headspace volatiles from heated trilinolenin (98% pure) have been analyzed via our microroom-GC/MS-computer system. Of over 100 collected volatiles, nearly 30 compounds were identified and their GC peak areas determined to contribute 80% to the total integrated chromatographic area. Primary volatiles from heated trilinolenin included 2-pentenal (5%), 2,4-heptadienal (22%), and 4,5-epoxy-2-heptenal (3%). Trilinolenin (triln)-8% mixed with tristearin (trist)-92%; a simulated soybean oil composed of trist-15%, triol-23%, trilo-54%, and triln-8%; and deodorized soybean oil were also subjected to heated oil volatile analysis.

Publications:

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KORITALA, S. AND E. N. FRANKEL. Selective Conjugation of Soybean Esters to Increase Hydrogenation Selectivity. J. Am. Oil Chem. Soc. 58 (1981):553-556.

MOUNTS, T. L., K. WARNER, AND G. R. LIST. Flavor and Oxidative Stability of Hydrogenated and Unhydrogenated Soybean Oil: Effect of Tertiary Butyl Hydroquinone. Am. Oil Chem. Soc. 58 (1981):792-794.

SNYDER, J. M., T. L. MOUNTS, C. R. SCHOLFIELD, AND H. J. DUTTON. Laboratory-scale Continuous Hydrogenation. Effect of Pressure. J. Am. Oil Chem. Soc. In press.

Other Reports:

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WARNER, K. Relationships of Flavor and Gas Chromatographic Volatile Analysis of Soybean Oil and Protein Products. Presented at American Oil Chemists Society Annual Meeting, New Orleans, LA, May 17-21, 1981.

SELKE, E. Analysis of Volatiles Derived from Materials Used to Produce U.S. Currency. Report No. 20910-081-1, March 9, 1981 (Not for Publication). Submitted to the Department of Treasury, Bureau of Engraving and Printing.

2. Quality Soybean Oil for Export Markets (E. N. Frankel)

a. Specific Objective: Evaluate critical quality factors of crude oil extracted from soybeans sampled from continuing export shipments to provide information as a basis for maintaining soybean quality during export.

Progress: Origin and destination samples were obtained from four identity preserved shipments of soybeans from Beaumont, Texas to Tillbury Grain Terminal, England. Crude oils were obtained by the usual procedure of cracking, flaking, and extraction with hexane. Quality analyses made on the crude oils included free fatty acids, iron, phosphorus, peroxide value, refining loss, and non-hydratable phosphatides. Origin samples were taken as composites while at the destination, samples were taken at various depths within the hold. Results showed that hold depth had no affect on oil quality deterioration. In general, free fatty acid, iron, and peroxide value showed no differences from origin to destination. However, destruction of the phospholipids was observed. The most marked deterioration in oil quality, occurred in refining loss. Beans at the destination yielded oils which had markedly higher refining losses than the same beans at the origin.

b. Specific Objective: Characterize factors of soybean storage as they affect crude oil quality.

Progress: A small adiabatic reactor was used successfully to simulate storage damaged soybeans. Four runs have been made; one to test the instrument, two to analyze the hexane-extracted oil, and one to be extracted by the $CO_2$ supercritical fluid method. Moisture content of all starting samples was 18% to 10%. Approximately 160 g of soybeans were used in each experiment. Intrinsic heating of the beans ranged from 45°C to 48°C and required 5 weeks for each run. Oil analyses of the two hexane extracted runs showed 3.07 and 2.49% FFA, 20 and 35 ppm total phosphorus, 0.1 and 0.17 ppm iron, and 0.0 and 0.99 peroxide value, respectively. A large reactor was constructed to hold approximately 2600 g (20% $H_2O$) of beans for further studies of non-hydratable phosphatides and supercritical $CO_2$ extracted soybeans.

Publication:

LIST, G. R., J. M. AVELLENADA, AND T. L. MOUNTS. Degumming of Soybean Oil : Effect of Operational Parameters on Lecithin Removal and Quality. J. Am. Oil Chem. Soc. 58 (1981):892-898.

Other Report:

BITNER, E. D., J. M. SNYDER, T. L. MOUNTS, H. J. DUTTON, AND G. BAKER. Monitoring Vegetable Oil Hydrogenation with an Analytical Chain. Presented at 1981 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 12, 1981.

3. Heavy Metals in Soybeans Grown on Sewage Sludge-Amended Soil (Cooperative Agreement - Western Illinois University)

a. Specific Objective: Investigate the effect on soybean yield and the modification of trace metal content of soybeans, meal, and oil from the use of municipal sewage sludge for fertilization.

Progress: Scientists at Western Illinois University grew experimental plots of soybeans on sludge-amended soil. Municipal sewage sludge was obtained from a rural community and used as a soil nutrient. The first year plan was four replications of plots 25 x 100 ft. each, with the following conditions for evaluation: (a) no added nutrients, (b) 46.5 tons/acre (dry basis) of sewage sludge, and (c) 93 tons/acre of sewage sludge. Soil analysis was performed to document soil type, and the nutrient concentration and heavy metal profile of 12 representative subsamples. Four representative subsamples of soybean plant tissue were taken 102 days after planting (pod-filling stage) for analysis of major elements, N&K; minor elements, Ca, Na, S, Mg, and trace elements, B, Cu, Fe, Mn, Zn. The latter four elements increased slightly in the treated soil as did the phosphorus and soil conductivity. Only the Zn was increased in the leaf samples taken. The yield of mature grain, 45 Bu/A, was reduced to nearly one-half with the high-level sludge application. This was generally attributed to abundant quantities of giant foxtail on the sludge treated plots. The second year plan was modified to provide for similar sludge application rates on 25 x 50 ft. plots thus allowing first year carryover effects of sludge fertilization to be determined. This proceeded as expected without drought injury, weed problems, or stunted plant growth.

## D. HUMAN REQUIREMENTS FOR NUTRIENTS

1. Biochemical Behavior of Isomeric Fats in Hydrogenated Soybean Oil (E. A. Emken)

a. Specific Objective: Interpret data and prepare manuscript on the incorporation of trans-13- and cis-13-octadecenoic acids in human lipids.

Progress: The absorption and distribution of deuterated cis- and trans-13-octadecenoic acid isomers (13c-18:1 and 13t-18:1) were compared to deuterated cis-9-octadecenoic acid (9c-18:1) in two young adult male subjects. A mixture of triglycerides was fed in a multiple-labeled experiment where each triglyceride contained a fatty acid labeled with a different number of deuterium atoms. Analysis of human plasma lipids by mass spectroscopy allowed the distribution of the two 13-octadecenoic acid isomers to be directly compared to cis-9-octadecenoic acid.

Chylomicron triglyceride data indicated all three fatty acids are equally well absorbed. Plasma data showed discrimination against incorporation of both the 13c-18:1 and 13t-18:1 isomers occurred in all neutral and phospholipid fraction. Discrimination against incorporation of the 13t-18:1 isomer into plasma cholesteryl ester and 2-acyl phosphatidylcholine was nearly absolute. The 1-acyl phosphatidylcholine fraction exhibited a large positive selectivity for the 13t-18:1 isomer. Differences for the relative distribution of the 13-18:1 isomers to 9c-18:1 in the various lipoprotein lipid classes were found.

b. Specific Objective: Complete human feeding studies and analysis of samples involving the trans-11- and cis-11-octadecenoic acid isomers in hydrogenated soybean oil.

Progress: Two subjects have been fed a mixture of triglycerides containing deuterated trans-11-, cis-11-, and cis-9-octadecenoic acids. Plasma and lipoprotein lipid fractions have been isolated, separated, and derivatized. These samples are being analyzed by mass spectroscopy. This work is in cooperation with Dr. Gulley, St. Francis Medical Center, Peoria, Illinois.

c. Specific Objective: Continue fat absorption studies in humans in order to provide baseline data for studies with children afflicted with cystic fibrosis.

Progress: The fatty acids which have been fed include stearic, oleic, linoleic, and elaidic acid which is formed during hydrogenation of soybean oil. Data for elaidic acid incorporation into plasma lipids resembles the stearic acid results more closely than oleic and linoleic acid results. A lipid conserving mechanism is apparent for the linoleic acid data. Three subjects have been fed to date and samples have been isolated and derivatized. Dr. Parson, our cooperator, has moved to the University of Calgary, Canada, and is establishing GC-MS facilities to complete the analysis of these samples at Calgary.

d. Specific Objective: Establish protocol for use of deuterium labeled fatty acids to measure the contribution of dietary fats to human milk lipids.

Progress: Devised experimental approach to use deuterium labeled fatty acids to follow the appearance of dietary fats in blood plasma lipids and milk lipids in lactating women. Results from our previous studies with adult male subjects were used to determine amounts of fats which need to be fed and frequency of blood sampling. Mass spectroscopy procedures used in our previous studies were used as basis for recommended analytical strategy.

This research will provide data on the nutritional requirements for lipids in lactating women and is in cooperation with Dr. Klein and Dr. Garza, Baylor College of Medicine, Houston, Texas.

e. Specific Objective: Design and plan human experiments to measure conversion of linoleic acid to archidonic acid and prostaglandins.

Progress: A protocol has been prepared which utilizes deuterium labeled linoleic acid to follow the rate of elongation and desaturation of linoleic acid to other long chain polyunsaturated fats such as arachidonic acid and dihomogamma linolenic acid. Strategies for designing these experiments considered the problems of placement of the deuterium label, analytical methodology for measuring blood lipids and prostaglandin levels, quantities of deuterated linoleic acid required, and sample collections. This research will be in cooperation with Dr. Judd of the Lipid Nutrition Laboratory, Beltsville, Md.

f. Specific Objective: Modernize mass spectrometer and computer systems to increase efficiency. Reduce down time and extend instrument capabilities.

Progress: Installation of the INCOS mass spectrometer data system is complete and most of its programs and procedures have been put in operation. The INCOS computer has been interfaced to the laboratories central Modcomp Classic computer using two parallel sixteen bit data channels, one for sending data and one for receiving data.

g. Specific Objective: Develop new techniques for determination of deuterium labeled compounds and analysis of lipids extracted from human blood.

Progress: New chemical ionization mass spectrometer techniques have been developed for the determination of labeled compounds and found to be about 10 times more sensitive then electron impact techniques. In actual practice electron impact is the more useful technique because the EI pattern of methyl stearate can be used to differentiate it from methyl oleate-$d_2$ and thus better handle methyl stearate interferences in real samples.

h. Specific Objective: Identify molecular structures using gas chromatography-mass spectrometry and other methods in collaboration with scientists from both within and outside NRRC.

Progress: Several surveys of naturally contaminated farm products have been run to determine the presence and quantity of 12 trichothecenes. Two computer programs have been written to handle the vast amount of data generated, one to produce mass chromatograms to highlight the mass numbers associated with the trichothecenes and the second program to do a general search for other possibly interesting compounds. High resolution peak matching still produces the best high accuracy mass numbers for use in formal proofs of structure.

i. Specific Objective: Complete feeding studies of trans-8- and cis-9-octadecenoic acids to laying hens.

Progress: Analyses of lipids from egg yolks and tissues of laying hens after feeding trans-8-octadecenoate-$^3$H(8t-18:1-$^3$H) and cis-9-

octadecenoate-$^{14}$C(9c-18:1-$^{14}$C) have been completed. trans-8-Octadecenoate was preferentially incorporated into only the phosphatidylethanolamines (PE), whereas discrimination against 8t-18:1-$^{3}$H occurred in the phosphatidylcholines (PC), triglycerides (TG) and cholesteryl esters (CE). The 1-acyl position of both PE and PC contained three times more 8t-18:1-$^{3}$H than 9c-18:1-$^{14}$C. Almost total exclusion of the 8t-18:1-$^{3}$H from the 2-acyl position of these phospholipids was found. Preferential incorporation of 9c-18:1-$^{14}$C occurred at the combined 1- and 3-acyl positions and at the 2-acyl position of yolk TG.

Tissue lipid analyses indicated that there was preferential deposition of 9c-18:1-$^{14}$C into all organs. Individual liver lipid classes displayed the same relative order of discrimination against 8t-18:1-$^{3}$H as did egg yolk lipids (CE>TG>PC>PE). These findings suggest that 8t-18:1-$^{3}$H was absorbed and catabolized more rapidly than 9c-18:1-$^{14}$C.

j. Specific Objective: Initiate feeding studies of trans-10-octadecenoic acid-$^{3}$H and cis-9-octadecenoic acid-$^{14}$C to laying hens to determine competitive distribution of these fatty acids into egg yolk and organ lipids.

Progress: Mixtures of methyl trans-10-octadecenoate-10(11)-$^{3}$H (10t-18:1-$^{3}$H) and methyl cis-9-octadecenoate-10-$^{14}$C (9c-18:1-$^{14}$C) were fed to three laying hens. Data show that only egg yolk phosphatidylethanolamines (PE) incorporated 10t-18:1-$^{3}$H to a greater extent than 9c-18:1-$^{14}$H. Other yolk lipid classes discriminated against the 8t-18:1-$^{3}$H in the order cholesteryl esters (CE)>phosphatidylcholines (PC)> triglycerides (TG). Acyl positional analyses indicate that the 8t-18:1-$^{3}$H was preferentially esterified at the 2-position of PE, but was discriminated against at TG 1+3, TG-2, and PE-2.

k. Specific Objective: Continue research to determine the distribution of dietary positional octadecenoic acid isomers in individual lipid classes in human tissues and initiate experiments to determine the relative oxidation rates of individual monounsaturated isomers by human heart tissue.

Progress: This study was designed to obtain information on the extent of incorporation and possible accumulation of fatty acid positional isomers in humans which occurs during long-term dietary consumption of hydrogenated fats.

Levels of trans-18:1 isomers have been determined for several individual lipid classes of human heart and liver. In general, neutral lipids were observed to contain higher levels of trans-18:1 than membrane phospholipids.

The double bond distribution in the cis and trans octadecenoic acid fraction of heart and liver phosphatidylcholine was determined. Isomers with double bonds near the methyl terminus were observed to accumulate slightly in tissues relative to isomers having double bonds

near the carboxyl terminus. In addition, the pattern of double bonds in the tissue provided evidence for specific metabolism of the cis-10 and trans-14-18:1 isomers.

l. Specific Objective: Complete evaluation of proposed synthesis of megatomoic acid [(E,Z)-3,5-tetradecadienoic acid] and its (Z,Z)-isomer and then prepare sufficient quantities (ca. 10 g) for evaluation in field tests by Dr. W. E. Burkholder (Stored Product & Household Insects Laboratory, USDA and Department of Entomology, U. of Wisconsin).

Progress: Wittig synthesis of 3-tetradecen-5-yn-1-ol gave almost totally the (Z)-isomer. Purification and reduction of this isomer gave (Z,Z)-3,5-tetradecadien-1-ol. Attempted oxidation of the dienol to the aldehyde gave largely carbonyl-conjugated aldehydes.

m. Specific Objective: Study various methods for the preparation of octadecatrienoic acid isomers.

Progress: Continue evaluation of synthetic procedure for utilizing sodium methoxide catalyzed interesterification of fatty esters with triacetin to form triglycerides. Preliminary results indicate good yields of triglycerides without isomerization of the triene isomers.

n. Specific Objective: Continue evaluation of synthetic schemes for preparation of deuterated octadecadienoate isomers.

Progress: Prepared sufficient linoleate-$d_4$ (<35 grams) for use as an internal standard in human metabolism studies and for use in cooperative research with the Lipid Nutrition Laboratory, Beltsville, Maryland.

o. Specific Objective: Synthesize and supply deuterium labeled fats for differential scanning calorimetry analyses to determine effect of the deuterium atoms on spacial orientation and thermodynamic properties.

Progress: Initiated cooperative study with Horticultural and Special Crops Laboratory (HSC). Provided Dr. J. Rothfus and Dr. S. Chang (HSC) with samples of methyl linoleate-15,15,16,16-$d_4$, 18:2-16,16,17,17-$d_4$ and 18:2-17,17,18,18-$d_4$, the four configurational (geometric) isomers of methyl 12,15 octadecadienoate 9,10-$d_2$ and methyl oleate-14,14,15,15,17,18-$d_6$.

p. Specific Objective: Synthesize triglycerides of the four geometric isomers of 9,12-octadecadienoic-15,15,16,16-$d_4$ acid for metabolic studies in humans.

Progress: Mixtures of methyl cis,trans- and cis,cis-9,12-octadecadienoate-15,15,16,16-$d_4$ have been prepared by the Wittig Reaction between hexyl-$d_4$-triphenylphosphonium bromide and methyl 12-oxo-cis-9-dodecenoate with butyl lithium in tetrahydrofuran. These geometric isomers were separated by partial silver resin chromatography and the 9c,12c-18:2 isomer was converted to the triglyceride for use in human metabolism studies.

q. Specific Objective: Minimize the formation of conjugated dienoates in the synthesis of methyl 9,12-octadecadienoates-$d_4$.

Progress: Methyl 9,12-octadecadienoates-$d_4$ are prepared by the Wittig Reaction between hexyl-$d_4$-triphenylphosphonium bromide and methyl 12-oxo-cis-9-dodecenoate. It was determined that the conjugated dienoates present in the 9,12-18:2 reaction mixture resulted from the presence of the conjugated aldehyde ester, methyl 12-oxo-trans-10-dodecenoate, in the cis-9 aldehyde ester used in the synthesis. This conjugated aldehyde ester is formed by isomerization of the cis-9-precursor during ordinary distillation or during silica gel chromatography. It was found that the isomerization could be avoided by distilling the aldehyde ester product mixture in a falling film molecular still. Analysis of the aldehyde ester mixture was complicated by the fact that the non-conjugated aldehyde ester conjugates partially during gas-liquid chromatography. Infrared spectroscopy allowed the analysis of these mixtures because in the non-conjugated aldehyde ester the aldehyde and ester carbonyl absorptions overlap at about 1740 $cm^{-1}$ while in the conjugated aldehyde ester two bands of equal and large intensity at 1740 $cm^{-1}$ and 1690 $cm^{-1}$ are obtained.

r. Specific Objective: Explore potential methods of synthesis of octadecatrienoate isomers.

Progress: Four different binary mixtures of the eight possible geometric isomers of methyl 9,12,15-octadecatrienoate were synthesized by the Wittig Reaction between cis- or trans-3-hexenyltriphenylphosphonium bromide and methyl 12-oxo-cis- or trans-9-dodecenoate butyl lithium in tetrahydrofuran.

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EMKEN, E. A. Use of Stable Isotopes in Human Metabolic Studies. Presented at the New Jersey Medical School, Newark, New Jersey, April 30, 1981.

EMKEN, E. A. Fate of Dietary cis- and trans-13-Octadecenoic Acids in Human Plasma. Presented at the 72nd Annual AOCS Meeting, New Orleans, May 17-21, 1981.

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## NORTHERN AGRICULTURAL ENERGY CENTER

M. O. Bagby, Manager

### A. BIOMATERIALS SCIENCE

1. Hydrocarbon-Producing Plants as Potential Multi-Use Crops (M. E. Carr)

   a. Specific Objective: Identify and call attention to plant species that are potentially adaptable to U.S. agricultural practice and have an overall composition favoring their economic development as multi-use botanochemical-producing crops.

      Progress: The NRRC Botanist collected 110 whole plant species mostly in central Illinois. Cooperating USDA plant scientists provided 22 species: six from Texas, 12 from Arizona, and four from Georgia. Whole plant samples representing 100 species primarily from the 1980 collection and from cooperating collectors were analyzed. Rhus glabra, Rhus copallina, Campanula pyramidalis, Phalaris arundinaces, Asclepias amplexicaulis, Asclepias sullivantii, and Arctium lappa contained 4 to 9% whole-plant oil.

   b. Specific Objective: Characterize oils, polyphenols, and hydrocarbon polymers produced by select U.S. plant species.

      Progress: Saponification of whole-plant oil extracts of Aleurites moluccana liberated 27.3% free acid; Aleurites trisperma, 22.2%; Asclepias amplexicaulis, 28.5%; and Asclepias sullivantii, 36.5%. Rubber extracted from Asclepias amplexicaulis and A. sullivantii has molecular weight of about 10 and 14% of that of Hevea. Thin layer chromatography of extracts from several leguminous whole plant oil species was utilized to fingerprint the composition and TLC Iatroscan analyzer identified a major component in an extract of Baptisia tinctoria as a wax ester.

   c. Specific Objective: Cooperate with university and USDA scientists in plant-breeding efforts to develop biomass crop varieties.

      Progress: Quack grass (Agropyron repens) samples (600) collected by a Minnesota USDA agronomist were analyzed for extractive variability. Total extractives ranged from 4 to 13% and less variability (0-6%) was observed for the hydrocarbon. Smooth sumac (Rhus glabra) plant samples, (100), were analyzed and the total acetone solubles ranged from 20 to 45%, and hydrocarbon content ranged from nearly 9 to 0.6%. 90 samples of bamboo (Phyllastachys sp.) from Georgia were analyzed and total acetone solubles ranged from 2.0 to 5.4%, and hydrocarbon content, (hexane fraction), ranged from 0 to 0.4%. 152 samples of sweet potato vines, [Ipomoea batatas (L.)] Sam., were recently received from Maryland for total oil and hydrocarbon analysis.

   d. Specific Objective: Evaluate various plant resins, oils, hydrocarbons for potential industrial utility.

Progress: Six species previously identified as having potential quantities of high energy extractables have been recollected and submitted to a university cooperator to determine gasification data, heating values and ash composition.

Publications:

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BAGBY, M. O. Renewable Sources of Liquid Fuels and Substitute Industrial Feedstocks. Invited presentation, Society of America Military Engineers, Illini Post, Champaign, Illinois, May 28, 1981.

BAGBY, M. O. Energy Alternative Source for Agriculture. Invited presentation, Great Plains Agricultural Council, Garden City, Kansas, June 9-11, 1981.

BAGBY, M. O. Energy-USDA Program. Invited presentation, 1981 USDA Sugarcane Harvesting Committee Field Tour and Meeting, Hilo, Hawaii, June 28 to July 2, 1981.

ROTH, W. B., I. M. CULL, B. S. PHILLIPS, C. L. SWANSON, AND M. O. BAGBY. Multi-Use Energy Potential from Whole Plant Oil and Hydrocarbon Analyses of Leguminosae Plants. Abstract. Joint Meeting of American Society of Pharmacognosy and Society for Economic Botany, Boston, Massachusetts, July 12-18, 1981.

2. Increased Energy Efficiency of Substrate Preparation for Alcohol Fermentations (R. W. Detroy)

   a. Specific Objective: Increase efficiency of saccharification of lignocellulosic residues to fermentable sugars.

Progress: Wheat straw modifications achieved by chemical, physical, and thermal techniques improved the conversion of cellulose to fermentable sugars by enzymatic hydrolysis. The saccharification efficiency of lignocellulose was increased with treatments that removed hemicellulose and/or lignin.

A two-step extraction of ground wheat straw with sodium hydroxide and sodium hypochlorite permitted 82-90% of the cellulose to be converted to glucose by cellulase. Hammer milling (-0.7 mm) of wheat straw improved its enzymatic hydrolysis fourfold. Thermal pulping of wheat straw provided a pentosan rich liquor with a pulp more susceptible to enzymatic attack.

Agricultural straw was subjected to thermal or alkali pulping prior to enzymatic saccharification. When wheat straw (WS) was treated at 170°C for 30 to 60 min at water-to-solid ratio of 7:1, the yield of cellulosic pulp was 70 to 82%. A sodium hydroxide extraction yielded a 60% cellulosic pulp and a hemicellulosic fraction available for fermentation to ethanol. The cellulosic pulps were subjected to cellulase hydrolysis at 55°C for production of sugars to support a C-6 fermentation. Hemicellulose was recovered from the liquor filtrates by acid/alcohol precipitation followed by acid hydrolysis to xylose fermentation.

Subsequent experiments have involved the fermentation of cellulosic and hemicellulosic hydrolyzates to ethanol. Apparently these fermentations were inhibited by substances introduced by thermal and alkali treatment of the straws, because ethanol efficiencies of only 40-60% were achieved.

Xylose from hydrolysis of wheat straw pentosans supported an ethanol fermentation by Pachysolen tannophilus strain NRRL 2460. This unusual yeast is capable of producing ethanol from both glucose and xylose. Ethanol yields were not maximal due to deleterious substances in the WS hydrolyzates.

b. Specific Objective: Investigate specific fungi/bacteria for amylase production for hydrolysis of raw starch to glucose.

Progress: Bacteria (20) and 18 fungi were screened (on soluble starch) for their ability to produce glucose. From these screens the following microorganisms were investigated for their ability to produce glucose from cracked corn kernels. Bacillus marcerans NRRL-B-430, B. subtilis NRRL-B-3696, B. subtilis NRRL-B-645; Aspergillus oryzae NRRL-468, A. awramori NRRL-3112, A. foetidus NRRL-337. Water soaked cracked corn (unbuffered) inoculated with A. foetidus routinely converted 18-22% crude starch to glucose in 3 days. B. marcerars converted 9-11% of the corn starch to glucose in similar experiments. All other microorganisms tested converted 1-5% of the corn starch to glucose within 3 days incubation at 25-28°C. Preliminary experiments indicate that glucose production from cracked corn can be increased by an additional 2-5% if the corn is presoaked in buffered solution for 24 hours prior to inoculation with the microorganisms. As judged by

glucose production, the microbial amylase activity apparently peaks at 3 days incubation and then gradually decreased to nil production after 7 days.

Publications:

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Numerous interviews for trade journals, television, radio, and newspaper releases.

3. Innovative Fermentation Technology for Alcohol Production (R. J. Bothast)

   a. Specific Objective: Design a novel process for the production of a high concentration of ethanol on a solid substrate.

      Progress: Initial experiments with Amylomyces rouxii on cracked corn showed that this microorganism would not grow at low moisture levels and that it was not very competitive. These properties are not commensurate with a practical process because of excessive contamination. Consequently, work with Amylomyces rouxii has been discontinued.

   b. Specific Objective: Evaluate selected microorganisms and conditions for increased alcohol yields from xylose.

Progress: The yeast Pachysolen tannophilus was found to be capable of converting D-xylose to ethanol. Batch cultures initially containing 50 g/liter D-xylose yielded 0.34 g of ethanol per gram of pentose consumed. Aerobic conditions were required for cell growth but not for ethanol production. Both alcohol formation and growth were optimum when incubation temperature was 32°C, when pH was near 2.5, and when D-xylose and ethanol concentrations did not exceed 50 g/liter and 20 g/liter, respectively.

c. Specific Objective: Determine the specific growth rate (m) and the specific product rate (v) for Zymomonas mobilis in a fed batch fermentation as a function of glucose and ethanol concentration.

Progress: A 10% inoculum of Zymomonas mobilis was added to an 8% glucose medium in a 30 liter fed batch reactor. After 4 hours, a 50% glucose feed was started and continued for 9 additional hours. In this reactor, 10% ethanol was produced with Zymomonas mobilis in 22 hours.

Most work needed to characterize a specific growth rate ($\mu$) and specific ethanol production rate ($q_p$) for Zymomonas mobilis as a function of temperature, pH, glucose, and ethanol concentration has been completed. Cells grow within 90% of maximum $\mu$ below 10% glucose, below 2% ethanol, between 30 and 37°C, and between pH 5 and 7. Growth ceases at approximately 15% glucose, approximately 8% ethanol, and at 40°C. Ethanol production rate decreased 20% as the percent glucose increased to 14%. As ethanol concentration increased from 1 to 15% at 4% glucose, specific ethanol productivity decreased linearly from 1.8 to 0.2 grams of ethanol per gram of cells per hour.

d. Specific Objective: Determine the feasibility of using immobilized microbial cells to convert sugar crops to ethanol.

Progress: Saccharomyces cerevisiae NRRL-Y-2034, S. uvarum NRRL-4-1347 and Zymomonas mobilis NRRL-B-806 each were separately bound in a Ca-alginate matrix and incubated in the presence of continuous free flowing and static glucose solutions of 1, 3, 5, 10, or 20% (wt./wt.) concentrations. In general, the continuous flow fermentation was superior in producing maximum ethanol yields over extended time periods. The immobilized yeast cells converted 100% of the 1, 3, 5, and 10% glucose solution to ethanol within 3 days and maintained this conversion rate for more than 2 weeks. The bacterium Z. mobilis converted 90% (wt./wt.) of these lower concentrations of glucose continuously for about 1 week. Both the yeasts and bacterium were inhibited in the high glucose (20% wt./wt.).

e. Specific Objective: Achieve high cell concentrations for the rapid conversion of sugars obtained from plant materials to ethanol in continuous fermentors.

Progress: The yeast Pachysolen tannophilus was entrapped in calcium alginate beads to ferment D-xylose on a continuous basis in the presence of high cell densities. Experimental operating variables included the

feed D-xylose concentration, the dilution rate, and the fermentor biomass concentration. Under favorable operating conditions, cultures retained at least 50% of their initial productivity after 26 days of operation. The specific ethanol production rate was dependent on the substrate level in the fermentor, passing through an optimum when the D-xylose concentration was between 28 and 35 g/liter. Consequently, reactor productivity increased with dilution rate and feed D-xylose concentration until a maximum was reached. The ethanol content of the effluent always decreased with increasing dilution rate, but excessive dilution rates diminished the ethanol content without increasing productivity. Unlike production rate, ethanol yield declined monotonicly from 0.35 g/g as the fermentor substrate concentration increased. The yield was 69% of that theoretically possible when the D-xylose concentration was near zero as opposed to 42% when it was in the range supporting the optimum specific rate of ethanol production. As long as D-xylose was supplied to cells faster than they could consume it, productivity increased with the mass of cells immobilized. The effectiveness factor associated with the calcium alginate beads used in this system was 0.4 indicating that only two-fifths of the entrapped biomass were effective in converting D-xylose to ethanol because of diffusion limitations.

f. Specific Objective: Determine the fermentability of feedstocks such as preserved high-moisture corn, ungerminated seed corn, sugar beets, etc.

Progress: Chemical preservation of high-moisture maize is one alternative to the conventional method of high-temperature drying and has contributed to increased use of high-moisture maize. The present study investigated the use of chemically preserved maize as feedstock for the production of alcohol by fermentation. Preservatives tested were formaldehyde, ammonia, sulfur dioxide, methylene-bis-propionate (MBP), acetic acid, and propionic acid. Acetic and propionic acids and ammonia treated maize samples were converted at all concentrations tested, with alcohol production at 80-90% of maximum theoretical alcohol possible. Sulfur dioxide treated maize yielded more alcohol than the other preservatives tested, when $SO_2$ treatments were kept at low concentrations (0.1-0.5%). MBP and formaldehyde treated maize yielded low amounts of alcohol and should be avoided as feedstocks for alcohol production.

g. Specific Objective: Investigate the potential of Zymomonas sp. to produce alcohol at high temperatures and sugar concentrations. (Compare the alcohol fermentation capacity of available strains of Zymomonas mobilis with that of yeast. Assess the advantages and disadvantages of selected strains of Z. mobilis for commercial alcohol production.)

Progress: Variants of all strains were isolated from 30% glucose medium which after a long lag period were fermenting. Plate counts from these fermentors onto yeast extract agar containing either 1% or 30% glucose indicated that most of the inoculated cells died off in the high glucose medium in the first 30 hours without producing $CO_2$. After 20 to 30 hours, a new population grew from what were apparently

a few high-glucose resistant cells. These high-glucose variants had the same short lag period to gas production as do the wild type cultures in low glucose medium. Strains B-14023, B-14022, and B-4490 and their high-glucose variants are better candidates for commercial alcohol production than the other strains. These can utilize 20 to 25% glucose completely with production of 9.5 to 12 weight % (12-15 vol.%) alcohol. There is no prolonged lag period in fermentation of high concentration of sucrose. Sucrose gives low yields of alcohol because part of the fructose is shunted into production of fructan. There is considerable variation in fructan production between strains of Z. mobilis as measured by absorbance at 600 nm after differential contrifugal removal of cells at 6000 R. C. F. When two lots of 25 liters of corn mash containing 0.2 bu. ground corn were fermented in 50 liter fermentors with Z. mobilis B-4490 and a wine yeast (Y-20340) both gave about 6.5 wt.% alcohol in about 40 hours. The same mash inoculated with B-4490 bu. ameliorated with 1800 grams of 60% dextrose syrup added 18 hours after seeding, produced 8.5% wt.% (10.6 vol.%) alcohol in about 40 hours. The same mash with the dextrose syrup added before inoculation with a "high-glucose" variant of B-4490 matched the yield and drop time of the delayed syrup addition fermentation.

h. Specific Objective: Screen specific yeasts/bacteria for ability to produce maximum alcohol from various fermentable sugars.

Progress: Cellobiose conversion screen in progress.

i. Specific Objective: Application of specific genetic technologies for improvement of alcohol production from plant polysaccharides by various fungi and bacteria.

Progress: Mitochondrial mutants (respiration deficient) and the wild type of Saccharomyces cerevisiae ID 41/161 were analyzed for rate of growth and alcohol production when grown under high/low (6.0/4.5) pH and high/low (10%/2%) glucose conditions. Regardless of the type of mitochondrial mutant (the portion of the mitochondrial genome retained), all mutants showed growth and alcohol production rates similar to those of the wild type under the same conditions. However, wild type cells retain the ability to respire alcohol when glucose becomes limiting while the mutants do not. Thus, mitochondrial mutants may prove useful in fermentation that extend over a period of time.

A number of nuclear mutants have been obtained (auxotrophic) by UV (ultraviolet) mutagenesin in Saccharomyces diastaticus (starch-degrader), and Pachysolen tannophilus (xylose-degrader).

Material derived from the acid hydrolysis of wheat straw by several methods inhibited the growth and formation of ethanol by P. tannophilus.

Ethanol tolerant variants of P. tannophilus have been isolated (growth in 5.5 vs. 3% EtOH). In some variants, ethanol production appears to be directly related to xylose utilization. In other variants, ethanol production continues 24-36 hours after all xylose has been utilized. These observations support the hypothesis that two or more reactions are involved in conversion of xylose to ethanol.

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BOTHAST, R. J. Alcohol Fermentation Developments. Presented at the 22nd Annual Corn Dry Milling Conference, NRRC, Peoria, Illinois, June 2-3, 1981.

BOTHAST, R. J. Innovative Fermentation. Presented at the Illinois Renewable Fuels Symposium, Dekalb, Illinois, June 26, 1981.

BOTHAST, R. J. Alcohol Research and Technology Update. Presented at the North Central Agricultural Experiment Cooperators' Conference, NRRC, Peoria, Illinois, October 19-20, 1981.

BOTHAST, R. J. AND R. W. DETROY. What is Alcohol? How is it Made? Presented at the Seminar on "Alcohol and Vegetable Oil as Alternative Fuels," April 7-9 (Raleigh, North Carolina), April 21-23 (Sacramento, California), and April 28-30 (Peoria, Illinois), 1981.

BOTHAST, R. J. AND R. W. DETROY. Innovative Fermentation. Presented at the Seminar on "Alcohol and Vegetable Oil as Alternative Fuels," April 7-9 (Raleigh, North Carolina), April 21-23 (Sacramento, California), and April 28-30 (Peoria, Illinois), 1981.

SILMAN, R. W. NRRC Alcohol and Solid Substrate Fermentation Research. Presented in a Seminar to the Chemical Engineering Department, Vanderbilt University, Nashville, Tennessee, April 13, 1981.

SILMAN, R. W., L. T. BLACK, AND K. NORRIS. Special Problems when Studying Solid-Substrate Fermentations. Presented at the 182nd National ACS Meeting, New York City, New York, August 23-28, 1981.

SLININGER, P. J. An Engineering Approach to Alcohol Production from Renewable Biomass. Presented at Bradley University, Peoria, Illinois, June 25, 1981.

SLININGER, P. J. New Yeast. Presented at a Workshop - Illinois Agriculture on "Conversion and Utilization of Biomass Feedstocks," Springfield, Illinois, September 14, 1981.

SLININGER, P. J., R. J. BOTHAST, J. E. VAN CAUWENBERGE, AND C. P. KURTZMAN. Conversion of D-xylose to Ethanol by the Yeast Pachysolen tannophilus. Presented at the First Engineering Foundation Conference on "Advances in Fermentation Recovery Process Technology," Banff, Alberta, Canada, June 7-12, 1981.

Numerous interviews for trade journals, television, radio, and newspaper releases.

4. Energy-Saving Methods for Recovery of Usable Protein from Alcohol or Methane Fermentation Media (J. S. Wall)

   a. Specific Objective: Continue work on composition of distillers' grains and compositions of residues from fermentations of other commodities such as grain sorghum and grain components such as corn flour.

      Progress: Grain sorghum was ground and fermented by a system similar to that of corn. The residue after fermentation was fractionated into distillers' grains, centrifuged solids and solubles. Sorghum distillers' grains have higher protein content and account for a bigger part of total grain protein compared with that of corn. Corn flour was fermented and the residue was separated into distillers' grains, centrifuged solids and solubles. Corn flour distillers' grains have much higher protein content but lower fat content compared with corn distillers' grains.

b. Specific Objective: Develop commercially feasible procedures for producing high protein-low fiber products from distillers' grains and diminish energy required in recovery of these products.

Progress: Corn distillers' dried grains with solubles (CDGS) and corn distillers' dried grains (CDG) at various moisture and fat contents were pin-milled and screened. Fractions of CDGS and CDG contained from 11.4 to 45.7% and 12.5 to 49.6% protein, respectively (dry basis), compared with 30% for CDGS and 25% for CDG. The best separations were obtained when CDGS at initial moisture of 21% was ground twice at 14,000 rpm and CDG at initial moisture of 21% was ground once at 14,000 rpm. The fractions with smallest particle size have highest protein, highest fat, but lowest dietary fiber contents compared with other fractions.

c. Specific Objective: Investigate re-use of stillage supernatant from centrifuged residual after fermentation of corn as media for subsequent fermentations to produce alcohol.

Progress: Variations in nature and amount of yeast inoculum fermentation were tested in several recycling experiments. With appropriate inoculum prepared by culturing the dry yeast for 24 hours, all glucose (20%) from the corn mash was consumed at 66 hours and levels of glucose above 9.0% W/V were maintained even when solubles were recycled 9 times. When alcohol was removed by steam distillation, the volume of stillage increased due to condensation. Suspended solids, mainly yeast was removed by centrifugation. After recycling 9 times, the weight of dried solubles increased from 2% to 6% of the solution. Minerals showed greatest increase in the stillage. Use of high pressure liquid chromatography to analyze the media and stillage established that glycerol was also produced in the fermentation and that glycerol accumulated in the recycled stillage solubles. Recycling of solubles solution from fermentation eliminates need for drying most of these solutions and provides considerable economics in costs and energy consumption.

d. Specific Objective: Develop low energy techniques for preserving moist distillers' grains and extending their usefulness.

Progress: Distillers' wet grains are valuable fermentation byproducts that are extremely perishable. Several chemical preservatives were evaluated as alternatives to energy-intensive, high-temperature drying of the wet grains. Sorbic acid, potassium sorbate, propionic acid, and ammonia at levels of 0.25%, 0.5%, and 1.0% were applied to 100-g samples of wet distillers' grains (61-67% m.c.) that were then held in a laboratory accelerated-storage apparatus. At the 1% treatment level, about 0.4 g of the dry matter was lost when the grains were treated with sorbic acid and held for 21 days. Dry matter losses averaged about 0.4 g when the grains were treated at the 1% level with potassium sorbate and ammonia and held for 9 days and 7 days, respectively. Propionic acid was ineffective in our tests. Storage of wet distillers' grains in a static carbon dioxide atmosphere held bacterial counts at initial levels for abut 8 days and molds and yeasts for about 16 days.

e. Specific Objective: Study the effect of processing conditions such as extrusion of grain, vacuum distillation of alcohol and enzymic or chemical treatment on distillers' feed grains on the feed values of the byproducts of alcohol production from distillers' grains.

Progress: Work in this area was not conducted due to limitations in personnel and high priority given to new work on food uses of distillers' grains. Corn distillers' grains, a high protein meal from fermentation of degermed and dehulled corn meal and low fiber dry-milled distillers' grains were acquired from commercial sources. These materials are being analyzed, and subjected to storage tests preparatory to incorporation into computer-formulated blended foods.

f. Specific Objective: Devise methods for fractionating, drying, and utilizing valuable feed materials from methane fermentation media.

Progress: Further studies on the effluent from the methane fermenter at the Meat Animal Research Center (MARC), Clay Center, Nebraska, produced slightly different results than previously reported. Wet sieving of the effluent, which contains 6% total solids (TS), isolates a fiber fraction representing 54% of TS. Low speed centrifugation (1500 X g) next isolates 26% of TS in a feed fraction leaving 19% of TS in the centrate. Dry sieving of the feed fraction did not yield subfractions with differing composition. Attempts at isolating useful materials from the centrate, which included biological oxidation, boiling at various pH values, and ultrafiltration, were not successful. Fifty-five percent of the centrate TS passes through a normal dialysis membrane. The material passing through a 60-mesh sieve is very similar to the centrate from the pilot scale centrifuge at MARC. This work has been completed.

Publications:

NOFSINGER, G. W., J. E. VAN CAUWENBERGE, R. J. BOTHAST, AND W. F. KWOLEK. A Preliminary Evaluation of Chemical Methods to Extend the Allowable Storage Time of Wet Distillers' Grains. Can J. Microbiol., in press.

WU, Y. V., K. R. SEXSON, AND J. S. WALL. Protein-Rich Residue from Corn Alcohol Distillation: Fractionation and Characterization. Cereal Chem. 58 (1981):343-347.

WU, Y. V. AND A. C. STRINGFELLOW. Corn Distillers' Dried Grains with Solubles and Corn Distillers' Dried Grains: Dry Fractionation and Composition. Submitted to J. Food Sci.

Other Reports:

NOFSINGER, G. W., R. J. BOTHAST, AND J. S. WALL. Fermentation Byproduct Recovery Processes--Recycling Solubles Solution and Chemical Preservation of Wet Spent Grains. Presented at the Feed and Fuels Symposium, Penn Center Inn, Philadelphia, PA, September 15-16, 1981.

WALL, J. S. Separation of Protein and Other Nutrients from Stillage. Presented at the Planning Seminar - Alcohol Production, Peoria, Illinois, January 19-20, 1981.

WALL, J. S., R. J. BOTHAST, A. LAGODA, Y. V. WU, R. A. ANDERSON, AND K. R. SEXSON. Maximizing Utilization of Distillers' Feeds and Other Byproducts of Alcohol Production. Symposium on Production and Conversion of Bioresources to Energy. Ag and Food Division, 181st National Meeting, American Chemical Society, Atlanta, GA, March 29-April 3, 1981.

WALL, J. S., R. J. BOTHAST, A. A. LAGODA, AND K. R. SEXSON. Effect of Recycling Distillers' Solubles on Alcohol and Feed Byproducts, Production from Grain Fermentation. Presented at the First Engineering Foundation Conference on "Advances in Fermentation Recovery Process Technology," Banff, Alberta, Canada, June 7-12, 1981.

WU, Y. V. AND A. C. STRINGFELLOW. Corn Distillers' Dried Grains with Solubles and Corn Distillers' Dried Grains: Dry Fractionation and Composition. Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June 7-10, 1981.

5. Physical/Chemical Modification of Vegetable Oils for Diesel Fuel (E. H. Pryde)

a. Specific Objective: Transesterify soybean oil with methanol to obtain methyl soy esters as part of a basic study to utilize these esters as diesel fuels.

Progress: The molar ratio of methanol/soybean oil has been varied to determine the minimum ratio which would yield a reaction product suitable as a diesel fuel. The molar ratios examined and the resulting weight percent of methyl soy esters obtained were: 6:1, 98%; 4.8:1, 94%; 4.2:1, 91%; 3.6:1, 87%; 3:1, 84%; 2:1, 69%; 1:1, 42%. The other products were glycerol and mixtures of tri-, di-, and monoglycerides. Sodium methoxide (0.5%) was the transesterification catalyst.

b. Specific Objective: Develop a quantitative method to determine the weight percent of methyl soy esters, tri-, di-, and monoglycerides in the above transesterification reaction mixtures.

Progress: By the use of the latroscan TH-10, an instrument which combines thin-layer chromatography with flame ionization detection, quantitation of the above compounds was achieved. Stearyl alcohol was used as an internal standard. Statistical analyses (W. F. Kwolek) of plots of weight and area ratios of compound/internal standard established linearity in the concentration ranges tested, and also defined a straight line equation for each compound. These data were then used to quantify the transesterification reaction products.

c. Specific Objective: Assist in technology transfer and development of basic information for the use of vegetable oils as emergency alternative fuels in crop production.

Progress: Assistance in planning and direct participation in the form of three lectures were provided for the three Regional Workshops "Alcohol and Vegetable Oil as Alternative Fuels" given in April and sponsored by the Agricultural Extension Service and the Northern Agricultural Energy Center. Similar assistance was provided for Seminar II - Vegetable Oils as Diesel Fuel, held at NRRC in October.

As a result of the Seminars held at the Northern Regional Research Center in 1980 and 1981, several conclusions have been reached which will expedite research on the vegetable oil fuel question. Among the conclusions are:

Vegetable oils can be used successfully in certain indirect injection, naturally aspirated, air-cooled diesel engines.

Vegetable oils cannot be used neat in direct injection diesel engines, which are the major power source for farm tractors in the U.S.

Blends of diesel oil with perhaps 20% vegetable oil can probably be used successfully in indirect injection engines.

Simple fatty esters perform better than triglycerides in the direct injection engines.

Numerous requests for information on vegetable oils and hybrid fuels from nationwide and international sources have been fulfilled.

Publications:

PRYDE, E. H. Vegetable Oil vs. Diesel Fuel: Chemistry and Availability of Vegetable Oils. Proceedings of Regional Workshops "Alcohol and Vegetable Oil as Alternative Fuels" 217-232.

PRYDE, E. H. Vegetable Oil vs. Diesel Fuel: Review of International Research. Proceedings of Regional Workshops "Alcohol and Vegetable Oil as Alternative Fuels" 287-295.

PRYDE, E. H. Vegetable Oil vs. Diesel Fuel: What Constitutes a Good Vegetable Oil Fuel? Proceedings of Regional Workshops "Alcohol and Vegetable Oil as Alternative Fuels" 297-304.

Other Reports:

BAGBY, M. O. Vegetable Oil as a Diesel Fuel - A Perspective. Presentation at Vegetable Oil as Diesel Fuel, Seminar II, NAEC, NRRC, Peoria, Illinois, October 21-22, 1981.

BAGBY, M. O. Vegetable Oils and Animal Fats as Diesel Fuels. Presented at Vegetable Oil as Diesel Fuel Seminar II, NAEC, NRRC, Peoria, Illinois, October 21-22, 1981.

PRYDE, E. H. Soybean Oil as Diesel Fuel Replacement. Presented at the Tri-State Soybean Forum, Vicksburg, Mississippi, January 9, 1981.

PRYDE, E. H. Vegetable Oils as Diesel Fuel. Presented at the Alternate Energy for Farms Shortcourse, Tifton, Georgia, January 27, 1981.

PRYDE, E. H. Vegetable Oil vs. Diesel Fuel: 1. Chemistry and Availability of Vegetable Oils; 2. Review of International Research; 3. What Constitutes a Good Vegetable Oil Fuel? Three talks presented for the Alcohol and Vegetable Oil as Alternative Fuels Regional Workshops April 7-9 (Raleigh, North Carolina), April 21-23 (Sacramento, California), and April 28-30 (Peoria, Illinois), 1981.

PRYDE, E. H. Vegetable Oils for Fuels and Chemicals. Presented at the 22nd Annual Corn Dry Milling Conference, Peoria, Illinois, June 2, 1981.

PRYDE, E. H. Potential of Vegetable Oil Fuels. Presented for the Survey Mission on Biomass Utilization Technology, Research Association for Petroleum Alternative Development, Japan Petroleum Institute, Peoria, Illinois, June 8, 1981.

PRYDE, E. H. Vegetable Oils for Fuel - An Overview. Presented at the North Central Agricultural Experiment Stations Cooperator's Conference, Peoria, Illinois, October 19-20, 1981.

PRYDE, E. H. Vegetable Oil Fuel Standards. Presented at the Vegetable Oil as Diesel Fuel - Seminar II, Peoria, Illinois, October 21-22, 1981.

PRYDE, E. H. Vegetable Oil Production and Processing for Fuel Use. Presented at the Society of Automotive Engineers Mississippi Valley Section Meeting, Dubuque, Iowa, October 22, 1981.

PRYDE, E. H. AND A. W. SCHWAB. Vegetable Oils as Diesel Fuel: Problems and Possible Solutions. Paper No. 168 presented at the Evald Skau Memorial Symposium, American Oil Chemists' Society Annual Meeting, New Orleans, Louisiana, May 17-21, 1981.

6. Long-Term and Endurance Engine Tests with Vegetable Oil Products as Diesel Fuel (Cooperative Agreement - North Dakota State University)

   a. Specific Objective: Investigate modifications or processing necessary to make vegetable oils satisfactory fuels for farm diesel tractors.

      Progress: Two endurance tests were completed on a diesel engine in the Allis-Chalmers Engine Division at Harvey, Illinois. The two tests included a 500 hour baseline test with #2 diesel fuel and a 600 hour test with a 50/50 blend of alkali refined sunflower oil with #2 diesel fuel. A continuous test cycle of 3 minutes at high idle, 2450 rpm, and 10 minutes at peak torque, 1925 rpm, was used. During the test on diesel fuel, there were no significant problems with the engine operation. However, problems were experienced while operating on the blended fuel, including plugged fuel filters, injector nozzle coking, excessive carbon deposits on the intake port, piston ring sticking, and operational problems with the turbocharger.

A new test program has been set up in accordance with the test cycle recommendations of the Equipment Manufacturers Association (EMA) Alternate Fuels Committee. Because of bad components, breakage of parts during shipment and delays in obtaining replacement parts for the electronic controller device required for the EMA cycle test, the scheduled programming of engine tests has been delayed several months. An extension of the Specific Cooperative Agreement of about 6 months will be required at no additional cost to the Agreement.

Reports:

KAUFMAN, K. R. Vegetable Oils as an Alternative for Diesel Fuel. Presented before the Oil and Fats Group meeting, Society of Chemical Industry, London, England, October 23, 1981.

KAUFMAN, K. R. Sunflower Oil as an Agricultural Diesel Fuel. Presented before the Agricultural Division/Institute of Engineers of Ireland and the Solar Energy Society of Ireland, Dublin, Ireland, October 27, 1981.

KAUFMAN, K. R. Vegetable Oils as an Alternative for Diesel Fuel. Presented at meeting sponsored by the Agricultural University and the Institute of Agricultural Engineering. Wageningen, The Netherlands, October 28, 1981.

KAUFMAN, K. R. Sunflower Oil and Methyl Ester as Fuels for Diesel Engines. Presented at the Third International Conference on Energy Use Management, Berlin, West Germany, October 30, 1981.

7. Model for Determining Feasibility of Potential Energy Crops (Cooperative Agreement - Purdue University)

a. Specific Objective: Establish critical factors that influence energy inputs and outputs for potential energy crops, and identify energy-efficient crop and process systems for production of liquid fuels.

Progress: Computer hardware has been ordered to link The Purdue University Computing Center with the NAEC Energy Research Information System. Software is being developed and input data for a variety of crops are being compiled.

Report:

PEART, R. M. AND J. R. BARRETT. Biomass Energy Research - Purdue and USDA, Peoria. Presentation at the Workshop on Advanced Biomass Technologies, Des Moines, Iowa, October 19-20, 1981.

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